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THE PATHOGENESIS OF TUBERCULOUS LEPTOMENINGITIS*

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INTRODUCTION

IN THE past, tuberculous leptomeningitis was generally thought to be due to direct hematogenous spread from some other tuberculous focus, whether this focus was in the stage of the primary complex, early miliary dissemination, isolated tuberculosis of a single organ or system of organs, or in the stage of late generalization (Kaufmann,[1] Austrian,[2] Beattie and Dickson,[3] Karsner,[4] and Brown[5]). According to this earlier view, only rarely was tuberculous leptomeningitis caused by direct extension from an older tuberculous lesion in the brain, dura mater or adjacent bone into the leptomeninges. Recently, Rich and McCordock[6, 7] presented the view which is supported by MacCallum,[8] Boyd,[9] and MacGregor, Kirkpatrick and Craig,[10] that tuberculous leptomeningitis is not caused by a direct hematogenous infection from a caseous focus in the body but by direct spread from an older lesion in the brain, meninges, or adjacent bony structure. The conclusions of Rich and McCordock are so important that further investigation seems to be warranted. With this view in mind, I have examined a series of patients with tuberculous leptomeningitis and of patients with tuberculosis in which the leptomeninges were not affected.

METHOD

After formalin fixation, forty-seven brains were cut into parallel slices which were not thicker than 3 mm. The brain slicing apparatus devised by Stevens[11] was used. The sections were transilluminated against a strong light

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and all suspicious areas found were removed for histologic examination. Often the slices were cut at right angles to prevent the oversight of very minute lesions. Sections for histologic study were taken in each case from the base of the brain and from all areas where lesions were suspected of lying in the cortex of the brain or close to the ependyma of the ventricles. Sections were also taken from the brains which did not reveal any macroscopic lesions.

RESULTS

In practically every case of tuberculous leptomeningitis, the exudation at the base of the brain was more abundant than over the remaining portions of the brain. Multiple tuberculomas were found more frequently than single

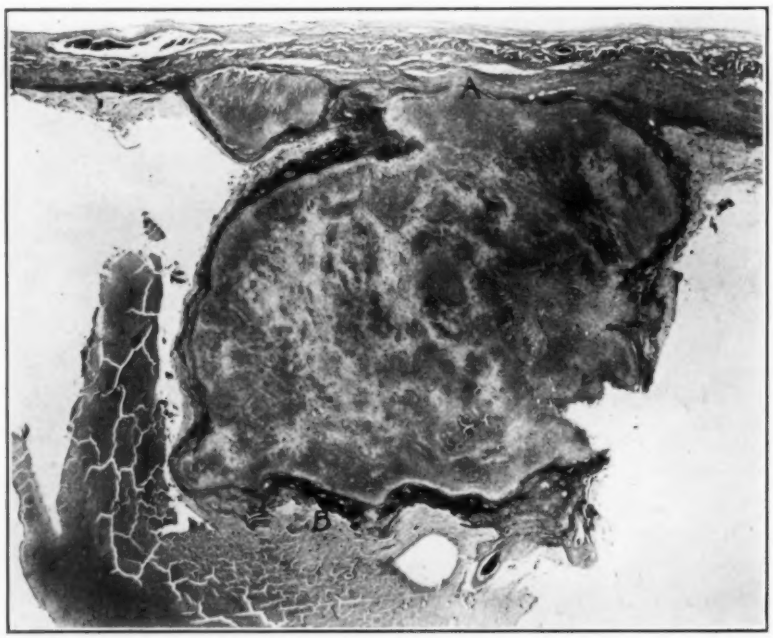

Fig. 1.—(P.M. 979, 1934.) Tuberculoma of the dura (*A*) extending and invading the cortex of the brain (*B*). Magnification ×11.

tuberculomas. Namely, six brains contained single tuberculomas, one of which was associated with an adjacent tuberculoma in the dura mater (Fig. 1), and thirteen brains showed multiple tuberculomas. Frequently, tuberculomas which appeared grossly to lie in the cortex of the brain, on histologic examination, proved to be located in the bottom of a sulcus.

On microscopic examination, a very marked perivascular infiltration of lymphocytes, epithelioid cells, and occasionally of polymorphonuclear leucocytes about the blood vessels of the pia mater was constantly found. These perivascular infiltrations extended along the smaller blood vessels and, to a lesser degree, often involved also the perivascular spaces of the cerebral cortex. In addition, subintimal infiltrations of lymphocytes and large histocytes, which separated the intima from the media, were often seen. Occasionally, the wall of a blood

vessel was partially or completely necrotic, or small subintimal tubercles were present. In one case, miliary tubercles were arranged about the wall of the small vessels.

When tuberculomas of the brain were found in close proximity to the leptomeninges, the adjacent meninges showed relatively little exudation, as compared to the extensive changes at the base of the brain. Miliary tubercles that on macroscopic examination were thought to occupy the cerebral cortex, in histologic sections often were found to be situated in the leptomeninges (Fig. 2), the cortex of the brain having undergone secondary softening due to a perifocal reaction.

The cases upon which my study is based can be arranged in 7 groups. Group 1 consists of 5 patients who ranged from twenty months to ten years of age and in whom the tuberculous leptomeningitis was associated with a

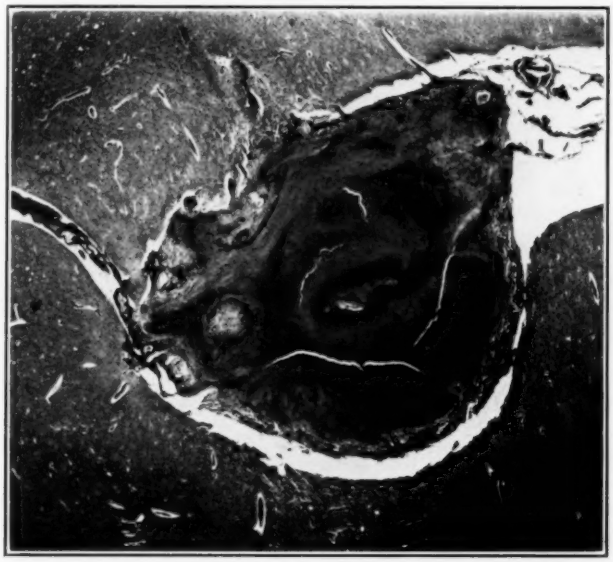

Fig. 2.—(P.M. 853, 1934.) Tuberculoma of the meninges lying deep in a sulcus of the brain which grossly appeared to lie in the cortex. Magnification ×22. Hemalum eosin stain.

progressive tuberculous primary complex. One patient showed a single tuberculoma of the brain and another patient showed multiple tuberculomas of the brain. In the 3 remaining patients no tuberculoma was found in the brain.

Group 2 is composed of 17 patients in whom the tuberculous leptomeningitis was combined with an early generalization of the tuberculosis. The age of these patients varied between five months and nineteen years. In this group there is 1 patient with a single tuberculoma and 4 patients with multiple tuberculomas of the brain. Twelve patients showed no tuberculous lesions of the brain.

In Group 3 I have placed 2 patients with tuberculous leptomeningitis who showed an isolated tuberculosis of a single organ or organ system. One of the 2 patients, aged twenty-nine years, had a single tuberculoma of the brain, and the other patient, aged forty-four years, showed no older tuberculous lesion of the brain.

The patients of Group 4 have tuberculous leptomeningitis associated with a late generalization. Of the 14 patients in this group, 1 had a single tuberculoma of the brain, 6 had multiple tuberculomas of the brain, and 7 had only miliary tubercles in the meninges.

Group 5 contains 4 patients with tuberculoma of the brain or spinal cord without tuberculous leptomeningitis. In this group, there is a patient with progressive tuberculous primary complex of the lung with multiple tuberculomas of the brain, a patient with tuberculoma of the spinal cord with isolated pulmonary tuberculosis, and 2 patients with late generalization with single tuberculomas of the brain.

Group 6 is formed by 4 patients with generalization in which the brain and the meninges are free from tuberculous changes. Three of the patients

Fig. 3.—(P.M. 633. 1934.) Large tuberculoma (*A*) in the cortex of the brain lying close to the surface, with the meninges (*B*) adjacent to it showing very little perifocal reaction. Magnification ×30. Hemalum and eosin stain.

with ages from five months to seven years belong to the early generalization, and 1 patient to the late generalization. The age of the later patient was seventy-four years.

Finally, Group 7 is represented by a patient in whom the only active tuberculous process was found to be multiple tuberculomas of the brain associated with a tuberculous leptomeningitis.

In summary, 39 of the 47 patients died of tuberculous leptomeningitis. All except 1 of these 39 patients were associated with an active tuberculosis outside the central nervous system. Of the 39 patients, 4, or 10.25 per cent, had single tuberculomas of the brain, and 12, or 30.76 per cent, had multiple tuberculomas of the brain, while 23, or 58.97 per cent, did not have any older tuberculous lesion of the brain or meninges.

Of the 4 patients with single tuberculomas of the brain, only 2 suggested a direct extension of the cerebral lesion into the leptomeninges, as indicated by

the amount of exudation in the leptomeninges which was perhaps greater about the tuberculomas in the brain than elsewhere in the meninges. In the other 2 patients, the leptomeninges adjacent to the tuberculomas showed but a slight perifocal reaction, whereas at the base of the brain the exudation was very marked, and in one case contained numerous focal areas of necrosis about the blood vessels.

Of the 11 patients with tuberculous leptomeningitis with multiple tuberculomas of the brain, only 4 showed a possible origin of the tuberculous leptomeningitis from the older lesion in the brain or, occasionally, in the leptomeninges. In the microscopic sections of these 4 brains, the amount of exudate in the meninges about the tuberculomas in the brain or meninges was distinctly greater or, at least, equal to that at the base of the brain. In the remaining

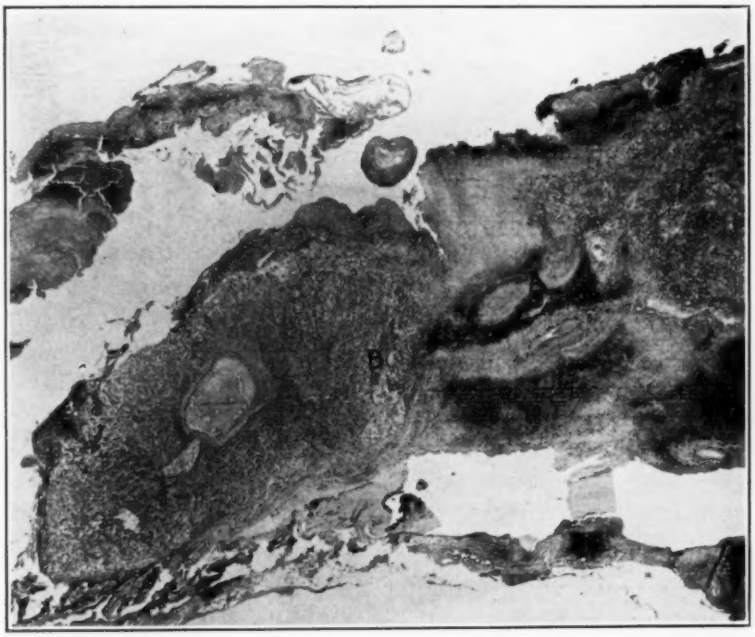

Fig. 4.—(P.M. 331, 1935.) Showing conglomerate tubercles (*A*) in the region of the posterior commissure extending into the pineal gland (*B*). Magnification ×17. Hemalum and eosin stain.

7 patients, the leptomeninges about the tuberculomas revealed only an insignificant perifocal reaction in comparison to the exudate at the base of the brain (Fig. 3).

One case demonstrated particularly distinctly the spread of the infection from an older lesion into the leptomeninges. In this case which concerns a colored woman, aged thirty-one years, the older lesion occupied the region of the pineal gland (Fig. 4). There were several other tuberculomas of the brain and a tuberculoma of the dura mater, but no active tuberculous lesion outside the brain or meninges.

Three cases of early generalization and one case of late generalization did not show microscopic evidence of tuberculous meningitis or older tuberculous lesions of the brain. Since permission was difficult to obtain, I was only able

to examine, in a few instances, the spinal cord. Of the cords examined only one was found to be the site of tuberculoma and in this case, the leptomeninges were free from tuberculosis. The other spinal cords showed only a recent tuberculous inflammation of the leptomeninges. Tuberculomas of the cord are rare. Thalhimer and Hassin[12] report 84 known cases in 1922. In most of the tuberculomas of the cord, there are symptoms simulating a tumor of the cord. Since 1922, Jennings[13] has cited 6 additional cases.

DISCUSSION

In the cases in which tuberculomas of the brain are found associated with tuberculous meningitis, the question arises as to whether the tuberculomas may have led to the tuberculous leptomeningitis, or whether the tuberculous meningitis is not related to the coexistent tuberculomas of the brain.

Rich and McCordock,[7] who have made an intensive study of 93 patients, 82 of whom died of tuberculous meningitis, state that the occurrence of meningitis as a result of the extension of infection from the so-called solitary and conglomerate tubercles in the brain substance is a well-recognized phenomenon. Cases of meningitis in which such an origin has been demonstrated have been decidedly exceptional in the experience of most of the earlier investigators. In their series of 93 cases, Rich and McCordock have found 66 patients in whom caseous foci of the brain did not discharge into the meninges and 13 patients with encapsulated quiescent foci of the meninges.

Even tubercles situated close to the meninges often fail to give rise to tuberculous meningitis. This phenomenon Rich explains on the basis of the infection of the brain substance during a period of bacillemia, which is known to take place during the progression of any visceral tuberculous lesion that occurs after resistance has once been acquired. In such a case, the tuberculoma will develop under the influence of some degree of resistance, and just as in the case of chronic adult phthisis, its progress will be retarded and the opportunity for connective tissue encapsulation increased.

Rich and McCordock state that when a diffuse miliary dissemination of the meninges occurred, they observed the presence of freshly caseous foci, containing many bacilli which arose near the meninges or ventricles and which did not exist long enough to become encapsulated. Of these older foci, Rich found 56 in the brain, 17 in the meninges, and 2 in both the brain and meninges. It was found necessary to cut the brain into thin slices in order to find these foci.

Radmann[14] in his recent report of 10 cases of tuberculous meningitis found that in three of the 10 brains there were multiple tubercles, varying from pinhead to cherry size, and in one brain he found only a single tubercle in the brain substance. It is important to note here that Radmann failed to find, both macroscopically and microscopically, the extension of the tuberculous process of the node in the brain substance into the meninges. In his series, the histopathologic changes in the meninges of the brains, having multiple tubercles, were much less than those changes found in the meninges where no tubercles or only a single tubercle was seen in the brain tissue. Despite these gross and histologic findings, Radmann agrees with Rich and McCordock that in these three cases tuberculous meningitis has been due to a local focus of dissemination

in the brain. He also states that in the brains without tubercles the tuberculous meningitis was produced by a direct hematogenous spread.

In analyzing Radman's cases, one cannot but be impressed by the fact that each patient had an active tuberculous process elsewhere in the body. Regardless of whether the active process was situated in the lungs or the lymph nodes, that process must be considered as a constant source of periodic or continuous discharge of tubercle bacilli into the blood stream, which may eventually lead to a tuberculous meningitis.

As stated before, in my series tuberculomas of the brain were found in 19 patients. Of these, 6 patients had single tuberculomas and 13 multiple tuberculomas. From a histologic study of the sections, in only 2 of the 6 patients with single tuberculomas was tuberculous meningitis attributed to progressive caseous tuberculomas of the brain. Of the other 4 patients, 2 had no meningitis and the remaining 2 had tuberculomas, around which no meningeal involvement manifested itself, whereas, in other areas, especially at the base of the brain, the tuberculous exudation was more marked.

Of the 13 patients having multiple tuberculomas of the brain, 2 patients did not have an associated tuberculous meningitis. Judging by the character of the adjacent meningeal exudate in only 4 of the remaining 11 patients could the tuberculomas be considered as immediate cause of the generalized leptomeningitis.

It is obvious that I did not find the small caseous foci of which Rich[7] speaks. In my patients all of the tuberculomas were of fairly large size. It was difficult, except in cases where the greatest amount of exudate surrounded the older caseating foci in the brain or meninges, to attribute the meningitis to these foci of infection.

Tuberculomas of the same age as those in the brain were also found in the meninges, thus denoting more of a diffusely disseminated development of tuberculomas. The finding of these tuberculomas leads one to question whether they were the result of a single massive infection with continued growth associated with central caseation necrosis (indicating an older lesion) or whether they were due to a confluence of miliary tubercles. As a whole, it was very difficult to decide this question in the advanced stages of the tuberculoma, but in a few of my patients, the confluence of miliary tubercles was apparent (Fig. 5).

Despite the fact that in this series most of the small and large tuberculomas did not have a well-developed connective tissue capsule, the adjacent leptomeninges revealed a relatively slight exudate.

The experimental production of tuberculous meningitis by way of the blood stream was found to be unsuccessful. Rich was not able to produce meningitis even by the injection of suspensions of tubercle bacilli into the carotid artery of rabbits or guinea pigs. In only a single animal did he find rare, minute, discrete tubercles, whereas, whenever he introduced them into the subarachnoid space, he invariably produced a tuberculous leptomeningitis simulating the human type. To compare experimental tuberculous meningitis produced by subarachnoid inoculation or by single or even repeated inoculation of tubercle bacilli via the blood stream with that of human tuberculosis is

hardly permissible, for, after all, in active human tuberculosis virulent organisms may be discharged into the blood stream over a considerable length of time.

Nagao[15, 16] and others, after injecting nonhemolytic streptococci or suspenpensions of India ink into the blood stream, failed to find them in the brain, spinal cord, and other surface organs but found that they were taken up chiefly by the phagocytic elements of the liver and spleen and, to some extent, by the lung.

Rich,[7] in his experimental work on tuberculous meningitis, showed that the changes about the blood vessels were identical with that found in human tuberculous meningitis, and that the exudate was also most marked at the base of the brain. The lesions about the blood vessels were characterized histologically by

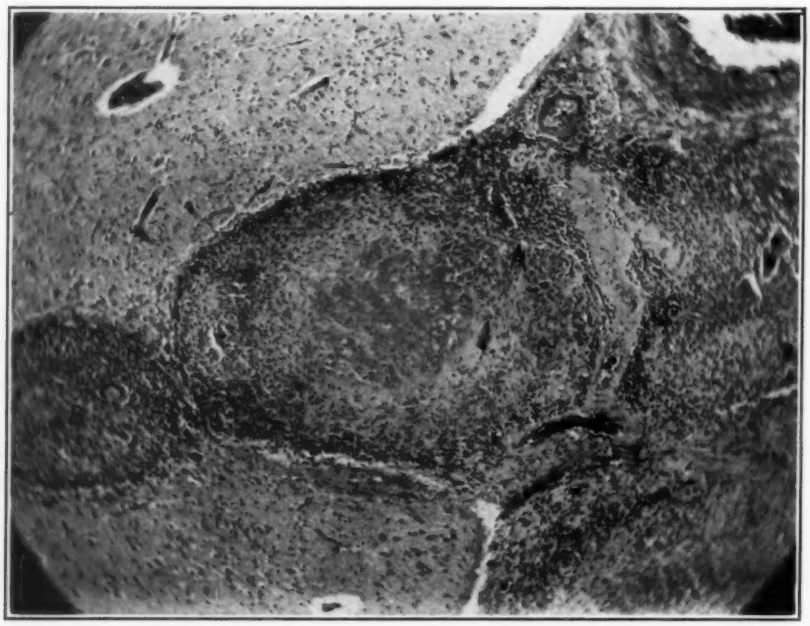

Fig. 5.—(P.M. 664, 1934.) Showing a confluence of miliary tubercle in the leptomeninges (*A*) and a single tubercle in the brain (*B*). Magnification ×100. Hemalum and eosin stain.

the marked infiltration and tubercle formation in the adventitia, and only later was there a subintimal proliferation. However, it is a well-known fact (Maximow and Bloom[17]) that blood vessels down to a diameter of 1 mm. have vasa-vasorum in the adventitia. One can readily see that the tubercle bacilli can easily be arrested in one of the fine capillaries of the adventitia and begin to proliferate, thus presenting a picture where the adventitia is involved before the intima.

Felton and Wegeforth,[18] in their study of the natural intrameningeal virulence of 21 varieties of bacteria injected into cats, found certain organisms, such as *B. coli, Bacillus paratyphosus B., pyocyaneous* and *Bacillus mucous capsulatus* to possess the greatest natural virulence for the meninges, and that when injected into the blood stream did not cause meningitis.

Weed and others[19] and Ayer,[20] using the same organisms as Felton and Wegeforth, were able to produce, experimentally, an acute meningitis by way of the blood stream, provided a withdrawal of spinal fluid was made at the height of the bacteremia or by any other method that caused a reduction of pressure of the cerebrospinal fluid. This reduction of spinal fluid pressure was also made by the intravenous injection of hypertonic salt solutions, by jugular compression, or by stoppage of the heart during such a bacteremia. Their control animals, receiving similar doses of the same bacteria, failed to develop meningitis, for the bacteria were promptly destroyed in the blood stream and the animals remained alive. They attributed this facilitation of invasion of the meninges by organisms circulating within the blood stream to alterations in the blood flow (passive and at times active hyperemia) through the cerebral vessels, with the reduction in the pressure of the cerebrospinal fluid as an important additional factor. The final pathologic picture was in no way different from the meningitis obtained by direct intracranial inoculation.

Upon examination of the visceral organs of the control and the experimental animals, Ayer[20] found similar, well-marked lesions in both types.

Another important point brought out by Ayer[20] is that the inner surface of the arachnoid serves as a limiting membrane and prevents the spread of the infection into the cortex of the brain. Whenever the infection occurred, it was infrequent and late in appearance.

A second and third defense are offered by a dense layer of connective tissue, which forms the inner portion of the pia, and by the molecular zone of the cortex, respectively, the latter having polymorphonuclear leucocytic infiltrations which withstand the invasion of bacteria.

From a clinical point of view, it has been repeatedly shown that in the greatest majority of patients dying of tuberculous meningitis, the disease is associated with either a generalized dissemination of tuberculosis or an active isolated visceral lesion.

Blacklock[21] states that in only a small percentage of 232 cases of tuberculous meningitis was the disease present in the absence of generalized miliary tuberculosis. Most of his cases occurred during the first year of life.

Kubo,[22] in his study, showed that 16 out of 23 cases of miliary tuberculosis developed basal tuberculous meningitis, and found that of the remaining 7 patients without macroscopic meningeal findings, 2 had minute meningeal tubercles widely separated from one another and situated exclusively about the arterioles or the capillaries of the leptomeninges, especially in the sylvian fissure. He maintains that there is no proof for the choroid plexus origin of tuberculous meningitis and that at most the infection occurs at the same time as the meningitis and is due to a primary hematogenous spread, which is contrary to the belief of Kment.[23] The latter maintains that the primary infection is in the choroid plexus and is followed by the development of the tuberculous meningitis, due to the spread of bacteria from the choroid plexus to the meninges.

In my series of 39 patients, dying of tuberculous leptomeningitis, all except one had a focus of visceral tuberculosis. Five cases were associated with a pro-

gressive primary tuberculous complex. Seventeen cases were associated with the stage of early dissemination, 2 cases with the stage of isolated tuberculosis in organs and organ systems, and 14 cases with the stage of late generalization.

SUMMARY AND CONCLUSIONS

This paper is based upon the study of 47 patients, 39 of whom died of tuberculous leptomeningitis, 23, or 58.97 per cent, of whom were definitely free of older lesions in the brain, meninges, or adjacent bony structures. Twelve patients, or 30.76 per cent, had multiple tuberculomas, of whom only 5 seemed to have been responsible for a tuberculous leptomeningitis. In the remaining 7 patients, meningitis was apparently the result of a direct hematogenous dissemination. Of 4 cases, or 10.25 per cent, of tuberculous leptomeningitis in brains containing single older tuberculomas, only 2 cases could be attributed to these older foci in the brain. Eight of the patients did not have tuberculous leptomeningitis, and 4 of these 8 had large tuberculomas situated in the brain tissue.

In totaling the number of patients with tuberculous leptomeningitis, 32, or 82.05 per cent, seemed to have been due to a direct hematogenous spread, and in only 7 patients, or 17.95 per cent, were there indications that older lesions in the brain or meninges had caused the diffuse infection of the meninges.

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NOTE.—Since this paper was submitted for publication my attention was called to a paper written by Ariel and Ssolowjew[24] who have shown that after repeated intravenous injections of India ink they found the pia mater to contain a few isolated cells filled with India ink. The same type of cell was found in the subarachnoid space, in the lumen of the meningeal veins and, occasionally, in the cellular space of the arachnoid.

They believe that the cells filled with India ink reached the pia and arachnoid membrane after wandering from the blood vessel into the meninges. In view of the fact that normally India ink is deposited in the meninges by way of the phagocytic cells, they suggest the possibility of certain microorganisms reaching the meninges in the same way.

BLOOD SEDIMENTATION RATES IN MIDDLE-AGED AND OLD PEOPLE*

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A PERUSAL of the literature reveals that little work has been done regarding the sedimentation rate in middle-aged and old people. We were interested in ascertaining whether the sedimentation rate would be a simple method of diagnostic value to us in our examination of the aged, since our laboratory facilities are limited, x-rays and detailed laboratory examinations being done at a different institution.

This work was done at the New York City Farm Colony, a home for the aged, with a capacity of 1,300 beds, of which 250 are infirmary beds. Six hundred and twenty-one sedimentation rates were taken on 496 apparently normal old men during the routine admission examinations. In this article, we are omitting the sedimentation rates obtained on our infirmary cases.

Our method consisted in drawing 3 c.c. of blood from a cubital vein and emptying it into a test tube containing potassium oxalate as the anticoagulant. Blood was drawn into 1 c.c. pipettes, calibrated into hundredths, and the fall of the cells at the end of an hour was read. The pipettes were placed in racks lined with a thick rubber base, to which they were firmly pressed by elastic bands, running from the rack to the top of the pipettes. We considered a drop of 10 per cent the upper limit of normal.

This work was started two years ago. Of the 496 men examined, 127 have left the institution, 57 have died at the Farm Colony, and 312 are still present.

This study concerns itself with older men, especially those over fifty years of age. Degenerative diseases are in the ascendancy in this group, and the sedi-

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mentation rate is not affected by degenerative changes. This is shown by Table I, where 66 per cent of our patients had rates under 10 per cent, and 86 per cent had rates under 20 per cent. Table II shows the rates of those who died; here also, 44 per cent had rates under 10 per cent, and 72 per cent had rates under 20 per cent.

Table I shows that age has little effect on the sedimentation rate. The slight variations present are due to the different number of cases in the various age groups.

The daily wear and tear of body tissue cause only slight changes in the rate, and it remains within normal limits. Tissue destruction, whether inflammatory or neoplastic in origin, causes an increase in the sedimentation rate. The test is not diagnostic of any disease, but an increased rate in an apparently

TABLE I

SEDIMENTATION RATES AND AGES OF ALL OUR CASES

AGES	SEDIMENTATION RATES						NO. OF CASES
	0-10%	11-20%	21-30%	31-40%	41-50%	61-70%	
35-39	2-100%						2
40-49	15- 83%	1-5.5%	1-5.5%	1-5.5%			18
50-59	63- 72%	16-18%	2- 2%	4- 5%	2- 2%		87
60-69	155- 64%	50-21%	17- 7%	13- 5%	5- 2%	3- 1%	243
70-79	78- 62%	24-19%	14-11%	5- 4%	3- 2%	1- 1%	125
80-89	11- 55%	6-30%	2-10%		1- 5%		20
90-99	1-100%						1
Totals	325- 66%	97-20%	36- 7%	23- 4%	11- 2%	4- 1%	496

TABLE II

SEDIMENTATION RATES AND AGES OF THOSE THAT DIED

AGES	SEDIMENTATION RATES						NO. OF CASES
	0-10%	11-20%	21-30%	31-40%	41-50%	61-70%	
50-59	2	3	1				6
60-69	13	8	5	3		1	30
70-79	6	5	3	1	1	1	17
80-89	4						4
Totals	25-44%	16-28%	9-16%	4-7%	1-1.5%	2-3.5%	57

normal person should be viewed with suspicion and call for further investigation. This was the basis for our use of the test.

We collected the following data on our malignancy cases. There were five cases of epitheliomas of the lip and face; six to twelve months previously, the sedimentation rates in these patients had been 26, 2, 12, 13, and 15 per cent. A patient wth carcinoma of the stomach had had a normal rate fourteen months before death; a second patient with gastric malignancy had had a rate of 33 per cent which had increased to 45 per cent eight months later, which was two months prior to death. One patient with malignancy of the rectum had had a sedimentation rate of 10 on two occasions, eighteen and fifteen months before the diagnosis was made. One patient with cancer of the biliary passages had a rate of 18, eighteen months prior to death. We had two cases of carcinoma of the prostate; one patient had had a normal rate fifteen months previously, the second

had had a rate of 20 per cent, eight months previously. The latter had pelvic metastases. A patient with carcinoma of the lung had a rate of 35 per cent, twenty-three months before death.

Two patients with silicosis gave rates of 17 and 12 per cent. Our highest rate was obtained in a patient in whom autopsy showed lung and kidney abscesses with generalized amyloidosis. Twelve patients with paralysis agitans showed normal rates.

Twenty-nine of our patients had diabetes mellitus, of whom 14 had a normal sedimentation rate, 6 a slight increase, 5 a moderate increase, and 4 a marked increase. The amount of hyperglycemia had no effect on the sedimentation rate.

Twelve patients with pulmonary tuberculosis showed normal to markedly increased sedimentation rates. All of our 5 patients with central nervous system syphilis had an increased rate ranging from 30 to 42 per cent. Five of 6 patients with tabes dorsalis had a normal rate, one had a slightly increased rate. Most of our patients with bronchiectasis had a normal or slightly increased rate.

In a series of 13 patients with diarrhea due to bacillary dysentery, the rates varied from 8 to 50 per cent. There were 2 patients with rates under 10 per cent, one with a rate of 15 per cent, 5 with rates in the twenties, 2 with rates in the thirties, and 3 with rates between 45 and 50 per cent. The average rate in these 13 patients was 29 per cent.

In the group of patients who died with rates under 10 per cent (25 in all), 18 or 72 per cent died of degenerative diseases of the heart, kidney, and vessels. One man died in an automobile accident, and 2 men died postoperatively (intestinal obstruction and transurethral prostatectomy). Two men died of malignancy, 1 of carcinoma of the rectum (one and a half years after the sedimentation rate was taken), and the other of carcinoma of the stomach (fourteen months after the sedimentation rate was taken). Three of the patients had tabes dorsalis.

In the group of patients who died with rates between 11 and 20 per cent (16 in all), 12 or 75 per cent died of degenerative diseases, 1 of bronchiectasis, 1 of silicosis, 1 of a malignancy of the bile passages (eighteen months after the sedimentation was taken), and 1 of acute bacillary dysentery. The 9 deaths in the group between 21 and 30 per cent were mainly due to degenerative diseases, 1 death being due to pulmonary tuberculosis. The 4 deaths in the group between 31 and 40 per cent included a ruptured aneurysm, carcinoma of the lung, carcinoma of the stomach, and generalized arteriosclerosis and myocarditis. The causes of death in the group with rates over 41 per cent (three patients) were central nervous system syphilis, abscess of the lung, and pyonephrosis.

DISCUSSION

This series of cases has been helpful to us in evaluating the use of the sedimentation test. A single test performed on a patient is not sufficient. The test only tells the presence of destructive changes at the time the test is done. Malignancies, when localized, do not cause an increased sedimentation rate; only when necrosis, secondary infection, and metastases occur are the rates increased. A single normal sedimentation rate does not rule out malignancy.

Remen found that 90 per cent of his 137 diabetic patients had an increased sedimentation rate. Kramer in a study of 366 diabetic patients found that 67.8 per cent showed abnormal readings. He also found that the blood sugar per se had no influence on the sedimentation rate. In our small group of 29 cases, 52 per cent showed an increase in the sedimentation rate.

Our tuberculous patients are mainly old fibrotic cases with negative sputa, and their sedimentation rates vary from normal to very high. One patient with a rate of 28 per cent advanced within six months to 45 per cent, at which time the sputum was Gaffky VI and the x-ray showed an advancement of the lesion. Our patients with syphilis, like those with tuberculosis, had rates varying from normal to very high. The tabetic patients had normal rates while those with central nervous system and cardiovascular involvement had increased rates.

SUMMARY

1. Sixty-six per cent of our patients had rates under 10 per cent, 86 per cent under 20 per cent.

2. Of our patients who died, 44 per cent had rates under 10 per cent, 72 per cent under 20 per cent.

3. Age has little effect on the sedimentation rate.

4. A single normal rate does not rule out pathology.

5. A high rate should be viewed with suspicion and should be cause for further study.

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THE FREQUENCY AND SIGNIFICANCE OF CHANGES OF THE EXPIRATORY CHEST VOLUME DURING ROUTINE MEASUREMENT OF BASAL OXYGEN CONSUMPTION*

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WITH TECHNICAL ASSISTANCE OF MURIEL WARD

WHEN measured by the closed circuit method with the most careful technic, a basal metabolic rate is frequently obtained which is higher or lower than is consistent with the clinical condition of the patient. Errors in the basal metabolic rate produced by factors within the patients themselves have been considered extremely rare. The escape of gas through either a dilated lacrymonasal duct or a patent eustachian tube with a perforated eardrum accounts for most of these. Changes of the expiratory chest volume which occurred immediately before or during measurement of basal oxygen consumption were found by Greene and Coggeshall[1] to produce significant errors in the calculated results. The assumption that the same quantity of air remained in the lungs at the beginning and at the end of each measurement of oxygen consumption was recognized by Benedict[2] to be the weakest point in the closed circuit method. He was convinced after a large number of experiments that this factor was insignificant in normal subjects. According to Carpenter,[3] changes in expiratory chest volume were insignificant in the earlier development of the method because the subjects were more or less trained, but became more significant when the subjects were unaccustomed to the apparatus. In fact, one of his subjects consistently added gas to the system instead of removing it.

Inasmuch as this source of error (changes in expiratory chest volume) has been ignored in the literature on clinical methods of measuring basal oxygen consumption, we have ascertained its frequency and significance in routine measurement of basal metabolic rate, and we have also noted the diseases in which the extreme changes may occur.

METHOD

Spirograms obtained during 2,985 routine measurements of basal oxygen consumption were examined for changes in the expiratory volume of the chest. The changes were manifested by alteration of the expiratory line of the spirogram and were classified into slight (1 cm. or less), moderate (1 to 2 cm.), and extreme (2 or more cm.).

We recognize that accurate measurement of changes in expiratory chest volume during measurement of basal oxygen consumption can be obtained only by simultaneous plethysmograms, but inasmuch as leaks in the system were ex-

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cluded during these spirograms, the degree of alteration of expiratory chest volume can be estimated from changes of the expiratory line. These estimations of chest volume are likely to be too small rather than too large. The final diagnoses of patients who exhibited extreme alterations of expiratory chest volume were arranged into groups.

RESULTS

Alterations of the expiratory chest volume occurred in 1,119 spirograms, or 37.5 per cent. Slight changes were present in 422, or 14.1 per cent, moderate

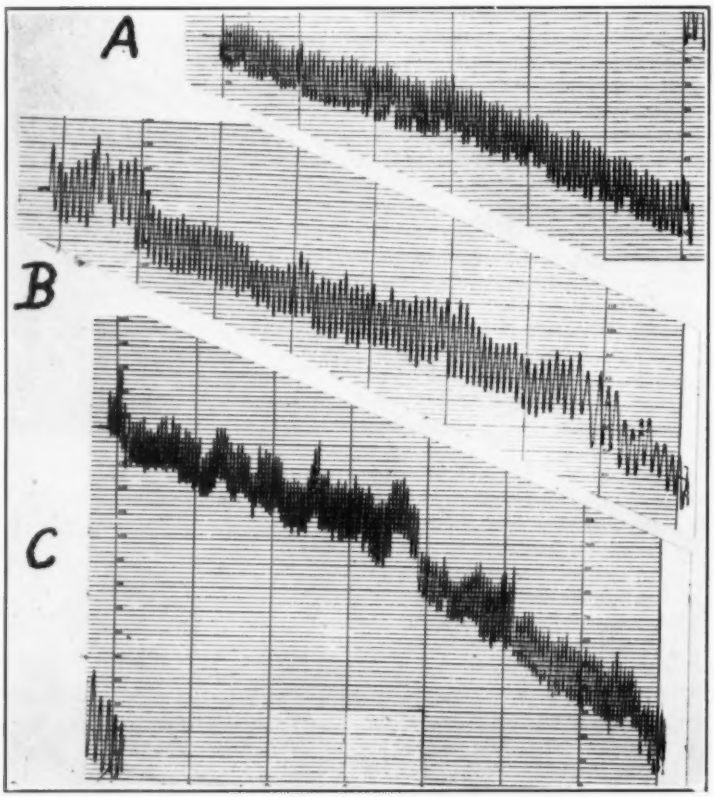

Fig. 1.—The three graphs show the more common changes in expiratory chest volume. *A*, slight; *B*, moderate; *C*, extreme.

in 603, or 20.2 per cent, and extreme in 94, or 3.1 per cent. All of the spirograms showing extreme changes and 198 of those showing moderate changes (a total of 292, or 9.78 per cent) gave variations in the calculated results of the basal metabolism from 8 to 45 per cent. The calculated results were not materially altered by any of the slight nor 405 of the moderate changes of chest volume. Alterations of the calculated results depend upon the abruptness and duration of the change in expiratory chest volume, whether or not the change occurs at either end or in the middle of the spirogram, and the quantity of oxygen consumed by the patient. The more common types of the slight, moderate and extreme changes are shown in Fig. 1.

It will be noted from Table I that the extreme changes in chest volume were not confined to any one disease or group of diseases.

TABLE I

DIAGNOSIS	NUMBER	DIAGNOSIS	NUMBER
Miscellaneous	14	Heart disease	6
Nontoxic adenoma thyroid	6	Obesity	2
Neurologic diseases	5	Anemia	4
Gynecologic diseases	10	Myxedema	7
Asthma	1	Graves' disease	6
Diabetes	3	Psychoneurosis	17

DISCUSSION

The customary requirements for basal state were strictly observed and, except in rare instances, a preliminary test was given the preceding day to acquaint the patient with the procedure. There were 2,750 of the spirograms which were obtained under the direct supervision of the same qualified technician. The frequency of changes in chest volume in the spirograms does not appear to be due to improper technic.

Although changes in chest volume usually induce errors which make the calculated basal metabolic rate too high, the reverse may occur and make the results too low. The only other recorded source of error which may falsely lower the calculated basal metabolic rate is the accumulation of carbon dioxide due to old soda lime, and this rarely, if ever, produces a significant underestimation of oxygen consumption. Lowering of the calculated basal metabolic rate by changes in chest volume is best illustrated by two cases recently observed. A white male who presented none of the symptoms or physical findings of myxedema persistently gave a basal metabolic rate of 38 to 40 per cent below normal. The chest was inflated immediately before the test and was slowly deflated while the spirograms were being obtained. When this source of error was eliminated the basal metabolic rate was 18 per cent below normal. The patient obtained no clinical improvement with thyroid medication. The basal metabolic rate reported in the case of a white woman with the typical clinical picture of Graves' disease was 15 per cent above normal. The same source of error described above was present, and when this was eliminated, the basal metabolic rate was 40 per cent above normal. The Graves' disease subsided following partial thyroidectomy.

Previous measurements of basal oxygen consumption do not exclude the occurrence of changes in expiratory chest volume. Ten of the 66 patients who exhibited extreme changes in chest volume had from 1 to 10 previous measurements and 6 of them had one or more satisfactory spirograms. This source of error, however, is usually not significant after the patient has become accustomed to the procedure. The number of measurements necessary for the patient to become accustomed to the procedure varies. Twenty-four of the patients exhibited changes in chest volume during the first measurement of oxygen consumption, but not in one or more subsequent ones. Satisfactory spirograms were obtained with 8 of the patients only after 2 or 4 attempts, and with 5 they were not obtained after four attempts.

Most of the changes in expiratory chest volume are easily discovered on the spirogram by the sudden onset and short duration. In our experience, practically all of the changes are detectable when one is aware that they occur and that they may be a source of error. The gradual changes are the most difficult to recognize. A majority of these, however, are the result of deflation and occur following a previous inflation which can usually be detected. Some patients continue throughout the course of six or even twelve to eighteen minutes with the expiratory chest volume increased above the normal level. This condition can be recognized by the occurrence of an occasional expiration which falls be-

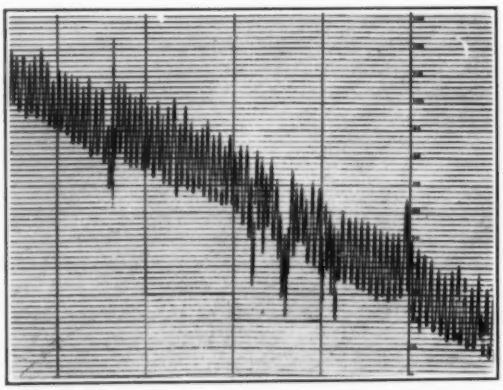

Fig. 2.—Showing the occasional expiration dropping below the average expiratory line.

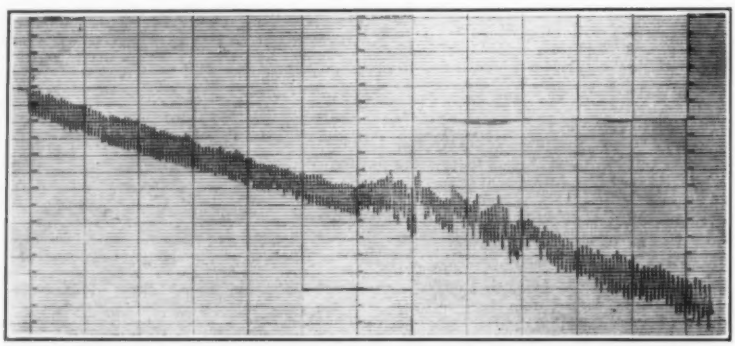

Fig. 3.—Showing extreme changes in expiratory chest volume during the first six minutes and practically no change in chest volume during the last six minutes of the spirogram.

low the average expiratory line as illustrated in Fig. 2. In our experience, this type of spirogram has been obtained only with an increased expiratory chest volume or with coughing.

Changes in expiratory chest volume can be eliminated as a source of error in approximately two-thirds of the cases in which it occurs by repetition of the procedure. In some cases the procedure must be repeated several times and in others it is necessary to continue the spirogram for twelve, eighteen, or even twenty-four minutes instead of the usual six or eight minutes without disconnecting the patient from the spirometer. It will be noted from Fig. 3 that a satisfactory spirogram was obtained during the second six minutes of the test.

The presence of changes of expiratory chest volume is of no diagnostic value, since it occurs in practically all diseases in which the basal metabolic rate may be measured.

The frequency of discrepancy between clinical picture and reported basal metabolic rate has practically disappeared since we have recognized and eliminated changes in chest volume as a source of error.

SUMMARY

The frequency of changes in the expiratory chest volume which occur during measurement of basal oxygen consumption by the closed circuit method has been ascertained. Changes occurred in 1,119 of 2,985 spirograms, or 37.2 per cent. Changes sufficient to alter the calculated basal metabolic rate significantly were found in 292, or 9.78 per cent. This error may produce an underestimation as well as an overestimation of the oxygen consumption. The methods of recognizing and eliminating this source of error are discussed. Discrepancies between the clinical condition of the patient and the reported basal metabolic rate are markedly reduced by recognition of this source of error. Change in expiratory chest volume may occur in any disease in which a measurement of basal metabolic rate is likely to be made.

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THE ACTION OF MAGNESIUM IN GUANIDINE INTOXICATION*

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INTRODUCTION

CALCIUM has been shown to protect the organism against the numerous pathologic manifestations produced by guanidine.[1-5] Führner,[6] working with an isolated nerve muscle preparation, showed that magnesium has a similar action to calcium in controlling muscle contractions caused by guanidine. This makes it interesting to know whether the many other manifestations of the intoxication seen when an intact animal is poisoned with guanidine can be controlled by magnesium. If this were true, the fact that large doses of magnesium can be given intramuscularly would make it a more convenient therapeutic agent than calcium to use in cases of clinical guanidine intoxication. Experiments were carried out to compare the effect of magnesium sulphate with that of calcium on the manifestations of guanidine intoxication.

COMPARISON OF PHYSIOLOGIC ACTION OF CALCIUM AND MAGNESIUM

The divalent ion magnesium may replace calcium in maintaining a suitable ionic equilibrium for certain biologic activities.[7] However, on certain other physiologic functions magnesium and calcium have an antagonistic action.[8] Although quantitatively different, the sedative effect of the two ions on the peripheral nervous system has been shown to be similar. It is well known that parathyroid tetany is due to a decrease in calcium and that the symptoms can be relieved by the administration of calcium.[9] Several investigators[10-12] have demonstrated that magnesium is also effective in preventing and controlling the symptoms of parathyroid tetany.

The antagonistic action of calcium and magnesium is best illustrated by their effects on the central nervous system. Meltzer and Auer[13] demonstrated the depressing effect of magnesium on the higher centers. They produced complete anesthesia in animals and showed that this anesthesia could be overcome immediately by the injection of calcium salts.[8] In fatal magnesium poisoning, Meltzer and Auer[13] demonstrated that respiration gradually becomes slow and shallow and stops before the heart action has ceased. This respiratory paralysis can be overcome and the function of the respiratory center completely restored by the administration of calcium. A further proof that calcium has a stimulating effect on the respiratory center is shown by the work of Hooker.[14] He perfused the medulla of dogs with saline containing red blood cells and introduced solutions containing first an excess of calcium ions and then potassium ions. The perfusing solution containing an excess of calcium ions stimulated the respiratory center.

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The action of calcium and magnesium on carbohydrate metabolism is not well understood. Lang and Rigo[15] produced a rise in blood sugar following the intravenous administration of moderate amounts of magnesium sulphate. Glycosuria following the intravenous injection of sea water was thought by Burnett[16] to be due to the presence of magnesium in the solution. Underhill[17, 18] used small doses of calcium and magnesium lactate subcutaneously and found that they failed to produce significant changes in the blood sugar content of rabbits, but the same amount of either salt intensified the effect of epinephrin on blood sugar. This suggests that the rise in blood sugar after the administration of small doses of calcium and magnesium is perhaps due to the adrenalin mechanism. Anesthetic doses of magnesium sulphate alone produce a marked increase in blood sugar.[18] Stewart and Rogoff[19] produced hyperglycemia following light ether anesthesia and asphyxia in adrenalectomized rabbits. This work suggests that the hyperglycemia produced by magnesium anesthesia is probably similar to the hyperglycemia produced by any other anesthetic and that the adrenalin mechanism has no part in it.

ACTION OF CALCIUM ON GUANIDINE INTOXICATION

Guanidine intoxication has been reported to occur clinically as a complication of various conditions[3, 20-24] and may be produced experimentally by the subcutaneous injection of guanidine hydrochloride. The symptoms of experimental guanidine intoxication are related chiefly to the nervous system and gastrointestinal tract. Nausea occurs early followed by vomiting and diarrhea. There is hyperexcitability of the nervous system which is manifested by restlessness, incoordination, and tremors which increase until the subject has generalized convulsions. This hyperexcitability of the nervous system is, at times, followed by profound depression. Chemically, there are a hypoglycemia and an acidosis. The hypoglycemia has been shown to be largely due to an inability of the poisoned animal to metabolize lactic acid in a normal manner.[2, 25] The lactic acid accumulates and leads to an upset in acid-base equilibrium. However, a tendency has been noted for the pH to decrease out of proportion to the decrease in alkali reserve and this would indicate a depression of the respiratory center by guanidine.[26]

Calcium has been repeatedly shown to protect the organism against the diverse manifestations of guanidine intoxication.[1-5] The lethal dose of guanidine is three or four times greater in animals kept on a high calcium diet than in subjects on an inadequate calcium intake.[1] The most efficient management of severe guanidine intoxication is to combine calcium therapy with other supportive measures, such as the forcing of fluids and the administration of glucose and an alkalinizing salt for the adjustment of the acid-base balance.[26] Nevertheless, intensive calcium therapy alone gradually decreases or abolishes the manifestations. The vomiting and diarrhea subside. The activity of the nervous system gradually becomes more normal and respiration improves, indicating increased activity of the respiratory center.[3, 4, 20, 27] There are an increase in the blood sugar and a decrease in lactic acid indicating a more normal metabolism.[1, 2, 5] These in turn tend to relieve the acidosis.

EXPERIMENTAL

Dogs which had been kept on a diet low in calcium for several weeks were poisoned by the subcutaneous injection of guanidine hydrochloride. When symptoms of the intoxication had developed, the animals were treated with magnesium sulphate. The following chemical determinations of the blood were made and the effect of magnesium on the objective nervous manifestations observed.

Blood sugar was determined by Folin's micromethod;[28] lactic acid by the method by Friedman and Kendall;[29] pH by the colorimetric technic described by Cullen;[30] CO_2 by the manometric method of Van Slyke and Neill;[31] guanidine by the colorimetric method of Major and Weber as modified by Minot and Dodd;[20] and serum magnesium by Brigg's colorimetric method.[32]

EFFECT OF MAGNESIUM SULPHATE ON BLOOD SUGAR OF ANIMALS WITH GUANIDINE INTOXICATION

Magnesium sulphate produced, as a rule, a rise in blood sugar when administered to dogs with hypoglycemia of guanidine poisoning. In Table I data are presented showing the rise in blood sugar and the different amounts of magnesium administered. The small doses were given intravenously and the larger ones intramuscularly and the time at which a dose was administered is shown in the table. The severity of the intoxication with guanidine varied in the different subjects as is indicated by the notes in the table. Likewise, the degree of hypoglycemia was not the same in all animals. In moderately severe intoxication the amount of magnesium sulphate administered had a definite influence on the amount of increase in blood sugar. For example, Dog M 19 which received 80 mg. of magnesium per kilogram of body weight, which, according to Meltzer and Auer[13] is about one-half an anesthetic dose, showed a greater rise in blood sugar than animals which received smaller amounts. In an overwhelming intoxication, as shown by Dog GB 6, magnesium only temporarily delayed the rapid fall of the blood sugar. So far as the blood sugar is an indication of the upset carbohydrate metabolism, magnesium acts similarly to calcium in restoring more normal conditions.

EFFECT OF MAGNESIUM SULPHATE ON THE ACID BASE BALANCE

Before studying the effect in intoxicated animals, one-half an anesthetic dose of magnesium sulphate was administered to normal dogs. This dose had been found most effective in raising the blood sugar of normal and intoxicated dogs and was selected because it was desirable to know whether an amount large enough to raise the blood sugar efficiently would have an untoward effect on the acid base balance of normal animals. The dose was divided and given in several different sites at one time and the effect on the pH, alkali reserve and lactic acid of the blood was studied. The maximum level of serum magnesium was usually reached at the end of one hour and then gradually came back to normal at the end of six hours when the experiments were terminated. The effect of magnesium on respirations was striking. Respirations became slow and shallow and continued so until the effect of the magnesium wore off. In Table II data are

TABLE I

EFFECT OF MAGNESIUM SULPHATE ON BLOOD SUGAR IN GUANIDINE INTOXICATION

DOG	TOTAL DOSE OF $MgSO_4$ MG. PER KILO	PRELIMINARY SAMPLE		BLOOD SUGARS FOLLOWING $MgSO_4$ THERAPY IN BROKEN DOSES MG. %							DEGREE OF INTOXICATION
		GUANIDINE MG. %	BLOOD SUGAR MG. %	½ HR.	1 HR.	1½ HR.	2 HR.	2½ HR.	3 HR.	6 HR.	
GB 3	19	1.84	61	*64	62	* 69	72	80	84		Moderate
GB 5	19	2.20	94	*93	99	*112	112	104	94		Moderately severe
GB 2	21	2.50	64	*99	98	*122	109	114	107		Moderate
GB 6	22	6.40	59	*60	66	* 45	47				Very severe
GB 4	23	2.43	52	*93	87	* 93	86	90			Moderate
GB 1	23	2.60	62	*59	71	* 76	99	73	81		Moderate
M 13	25	1.16	89	*	103		98		96	90	Mild
M 3	28		56	*		*120					Severe
M 11	45	5.90	118	*	138	*	154				Severe
M 19	80	4.80	85	*			130			194	Moderately severe
M 20	80	4.64	56	*			86				Severe

*Indicates time interval of administration of broken doses of magnesium sulphate.

TABLE II

EFFECT OF ONE-HALF ANESTHETIC DOSE OF MAGNESIUM SULPHATE ON THE ACID BASE EQUILIBRIUM OF NORMAL DOGS

DOG	TOTAL DOSE OF $MgSO_4$ MG. PER KILO	PRELIMINARY SAMPLE				1 HOUR AFTER DOSE				2 HOURS AFTER DOSE				3 HOURS AFTER DOSE				6 HOURS AFTER DOSE				REMARKS
		pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	
M 12	80	7.35	53.0	18.4		*7.28	43.5	45.2		7.28	40.9	55.1		7.33	38.2	65.0		7.38	50.1	9.9		Dog very drowsy and lay on floor but could be aroused
M 12	80	7.38	51.9	12.7		*7.19	42.6	29.7		7.20	37.2	56.5		7.23	37.6	42.4		7.35	49.6	18.3		Dog very drowsy but could be aroused
M 18	80	7.40	46.1	11.3	2.4	*7.39	44.3	16.9	12.2					7.37	43.9	8.1	11.2				6.0	Marked drowsiness but was conscious at all times
M 14	80	7.36	49.2	19.8		*7.29	43.3	38.1		7.29	48.8	28.2		7.33	47.9	19.8		7.31	47.2	16.9		Quiet when left alone but could be aroused
M 15	80	7.40	54.8	16.9		*7.28	43.3	36.7		7.27	45.2	36.7		7.27	45.2	36.7						Very quiet but conscious Respirations irregular
M 16	80	7.39	53.8	26.8	2.2	*7.28	40.3	60.8	14.5	7.26	35.9	62.2	14.4	7.24	39.6	50.9	13.2	7.38	43.4	11.3	5.6	Quiet but conscious
M 17	80	7.39	46.5	19.8	2.0	*7.30	37.8	36.7	13.9					7.36	44.9	24.0	7.1					Depressed but could be aroused

*Indicates time of administration of a single dose of magnesium sulphate.

presented showing the effect of magnesium on the acid base balance of the blood. The pH was decreased by about 0.05 to 0.18 while the alkali reserve was slightly decreased and the lactic acid considerably increased. This decrease in pH without a marked fall in alkali reserve points to a depression of the respiratory center which has brought about an accumulation of free CO_2 in the blood. The accumulation of lactic acid is probably a result of poor oxidation as a result of decreased respirations. The increase in lactic acid probably accounts for the slight decrease in alkali reserve. Since this dose of magnesium depresses the respiratory center of normal animals the prospect of its safe administration to animals with a pH already below normal is poor. However, the effect of magnesium sulphate on the acid base balance in guanidine intoxication was studied.

Guanidine hydrochloride was given subcutaneously to the dogs and the experiments started whenever acidosis developed. The lactic acid was high in all of the animals. The level varied with the degree of hyperexcitability and the restlessness and was usually higher in animals having convulsions than in those which were quiet. Sometimes, however, equally high concentrations of lactic acid were found in subjects showing no increase in muscular activity. These results are in accord with previous work which has demonstrated the inability of intoxicated animals to metabolize lactic acid in a normal manner.[2, 25] The dose of magnesium administered varied from a small amount equivalent to the dose of calcium which we have used in the treatment of guanidine intoxication[3, 20, 21, 26] to half an anesthetic dose or from 19 to 80 mg. per kilogram of body weight. The small doses were given intravenously and the larger ones intramuscularly. The time at which a dose was given is shown in the table. The data in Table III are presented in the order of ascending doses of magnesium.

Small intravenous doses of magnesium had very little effect on the pH of the blood. Immediately after the injection the animals usually showed some depression of respiration but no change in the pH of the blood was noted in the sample collected one hour after the administration. However, when larger doses of magnesium were administered a more prolonged depression of respiration was observed. When the dose of magnesium reached the level of 25 or 30 mg. per kilogram, a decrease in the pH of the blood was noted. This fall in pH became more marked as the dose gradually increased to 80 mg. per kilogram. Soon after receiving a single dose of magnesium and before chemical studies of the blood were made, a few experimental animals died. Their death appeared to be due to respiratory failure, because respirations ceased before the heart stopped. These incomplete experiments are not included in the table, but are mentioned because they serve to strengthen the impression that the use of magnesium is limited by its depressant effect on the respiratory center. A decrease in lactic acid was observed with the larger doses of magnesium. This decrease appears paradoxical when compared with the increase in lactic acid observed in the group of normal animals. It must be recalled, however, that some animals, poisoned with guanidine, show convulsive symptoms. This muscular activity greatly increases the rate of production of lactic acid. This excess production of lactic acid in an animal with a defective lactic acid metabolism accounts for the initial high lactic acid content in the blood of intoxicated animals. With the

TABLE III

EFFECT OF MAGNESIUM SULPHATE ON ACID BASE EQUILIBRIUM OF DOGS INTOXICATED WITH GUANIDINE

DOG	TOTAL DOSE OF $MgSO_4$ MG. PER KILO	PRELIMINARY SAMPLE					1 HOUR AFTER FIRST DOSE				2 HOURS AFTER FIRST DOSE				3 HOURS AFTER FIRST DOSE				6 HOURS AFTER FIRST DOSE				DEGREE OF INTOXICATION	REMARKS
		GUANIDINE MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %		
GB 3	19	1.84	7.13	18.0			*				*				7.14	22.0							Moderate	During injection respirations became deep and slow. Dog showed few twitching movements of legs after $MgSO_4$.
GB 5	19	2.20	7.29	37.0			*				*				7.29	39.1							Moderately severe	After $MgSO_4$ therapy dog looked better. Stood up and drank water.
GB 2	21	2.50	7.08	29.7			*				*				7.18	36.1							Moderate	During injection color became very pink and respirations were rapid and irregular.
M 72	22	2.56	7.24	35.3	22.9		*				7.28	29.0	20.9										Moderate	Given $MgSO_4$ intramuscularly along with guanidine. When acidosis had developed was given $MgSO_4$ subcutaneously. No noticeable change after $MgSO_4$ therapy.
GB 1	23	2.60	7.29	36.8			*				*				7.10	33.5							Moderate	Did not look improved after intravenous $MgSO_4$.
GB 4	23	2.43	7.33	31.4			*				*				7.31	29.8							Moderate	Mucous membranes very pink and breathing irregular during injection. No improvement after therapy.

TABLE III—CONT'D

M 32	23	1.39	7.25	26.0	67.8	2.60	*7.25	28.3	38.2	7.10	*7.25	24.3	36.7	8.9	*7.25	26.3	48.0	12.3					Moderately severe	Convulsive movements of legs stopped after $MgSO_4$ and respirations were slow. Became very quiet. Died one and one-quarter hours after second dose of $MgSO_4$.
M 31	24	5.45	7.25	28.8	49.5	3.90	*7.23	23.5	63.6	13.0	*7.26	26.6	41.0	16.2									Severe	After first injection dog became very stuporous and respirations were slow and deep. One hour after second dose respiration stopped before heart stopped.
M 13	25	1.16	7.33	36.1	33.9		*7.32	34.3	42.1		7.37	36.4	38.2		7.37	40.9	15.5		7.34	41.0	12.7		Mild	During intravenous injection of part of dose respirations became irregular. No noticeable change in general condition after $MgSO_4$.
M 3	28		7.18	26.4			*7.03	24.9															Severe	Received $MgSO_4$ intramuscularly along with guanidine. Probably experiment started with high serum Mg. Vomited during injection of intravenous $MgSO_4$. Became relaxed for few minutes, then twitching movements started. Experiment discontinued at end of one hour.
M 5	28	3.20	7.24	33.5			*				7.22	29.5			*				5 hr. 7.14	28.9			Moderately severe	$MgSO_4$ along with guanidine. Became quieter after intravenous $MgSO_4$. No improvement in general condition.

TABLE III—CONT'D

DOG	TOTAL DOSE OF $MgSO_4$ MG. PER KILO	PRELIMINARY SAMPLE					1 HOUR AFTER FIRST DOSE				2 HOURS AFTER FIRST DOSE				3 HOURS AFTER FIRST DOSE				6 HOURS AFTER FIRST DOSE				DEGREE OF INTOXICATION	REMARKS
		GUANIDINE MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %	pH	ALKALI RESERVE VOL. %	LACTIC ACID MG. %	SERUM MG. %		
M 30	31	2.95	7.23	22.2	132.0	3.10	*7.11	20.2	67.9	9.20	*												Severe	After injection of $MgSO_4$ twitching movements of legs stopped. Died ten minutes after second dose of $MgSO_4$.
M 11	45	5.90	7.22	25.7	69.3		*7.04	24.0	56.5		*7.09	22.6	19.8										Severe	Became quieter after $MgSO_4$. Breathed slower. Experiment discontinued at end of two hours.
M 7	65		6.97	12.5	166.3		*				6.97	15.5	135.0		*7.00	4 hr. 20.2	82.0		*5 hr. <6.95	21.6	89.1		Severe	$MgSO_4$ given intramuscularly. Did not improve after therapy and continued to have twitching movements of muscles. Died 1 hour after third dose $MgSO_4$.
M 19	80	4.80	7.24	29.2	72.1	3.30	*				7.14	23.8	70.7	19.9					7.05	21.9	82.0	20.1	Moderately severe	Less convulsive movements after $MgSO_4$. General appearance unimproved. Did not void during experiment.
M 20	80	4.64	7.29	42.6		2.90	*				7.15	36.8		28.9									Severe	Quiet for one and one-half hours after dose. Then twitching movements became severe. Died two and one-half hours after dose of $MgSO_4$.

*Indicates time interval of administration of broken doses of magnesium sulphate.

larger doses of magnesium sulphate, convulsions are stopped and the production of lactic acid decreased. This must be one factor in causing a decrease in lactic acid. On the other hand, the action of magnesium may possibly be similar to that of calcium in making it possible for the poisoned animal to metabolize lactic acid in a more normal manner. Balanced against this possibility is the fact, which was demonstrated in normal animals, that larger doses of magnesium may so depress respiration that the intake of oxygen is inadequate. The variability in the effect of magnesium on the lactic acid in intoxicated animals probably depends on which of these various factors predominate in a given experiment.

COMPARISON OF OBJECTIVE NERVOUS MANIFESTATIONS

The "remarks" in the foregoing tables indicate that the most outstanding objective change in intoxicated animals following magnesium therapy is the control of convulsive symptoms. The animals become quiet and relaxed and respirations are slow and shallow. Following calcium medication, there is also a relief of hyperexcitability but less evidence of a depression of the higher centers. The animal is quiet but alert and respirations are normal in character. Repeated medication with the two therapeutic agents further accentuates the objective difference in appearance. The dog treated with magnesium becomes progressively depressed and finally dies while the one receiving calcium tends to improve progressively.

CONCLUSION

As a result of these studies, it appears that on those functions in which calcium and magnesium have a synergistic effect in the normal animal magnesium offers a protection similar to calcium against guanidine intoxication. Both raise the blood sugar and quiet the peripheral nervous manifestations. On the other hand, in the control of disturbances caused by guanidine on the higher centers on which calcium and magnesium have an antagonistic action, magnesium cannot replace calcium. The benefit derived from magnesium in quieting the peripheral nervous manifestations and raising the blood sugar is counteracted by the depressant effect on the respiratory center.

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PAIN AND WEATHER*

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THE question of whether there is any relationship between the occurrence of pain in man, and weather changes, is a very interesting and important one, and it is strange, considering the amount of good that could come from a knowledge of the cause of the recurrence of pain, that so pitifully little has been done in its investigation. The meager work that has been done along these lines has dealt mostly with weather changes and arthritic pain. In 1877 Mitchell[1] reported on data collected from the records of an army officer suffering from neuralgia pain following amputation of a leg. He made daily observations for 3.5 years and maintained he could forecast the weather by the attacks of neuralgic pain that seized him just before the onset of storms. He concluded as a result of an analysis of these data that separate factors of storms, such as lessened pressure, rising temperature, greater humidity, and winds, appear as a rule as incompetent when acting alone to give rise to attacks of pain. Either then, it is the combination that works the mischief, or else there is in times of storm some as yet unknown agency productive of evil. Such an agent may be either electricity or magnetism. In 1878 Everett[2] reported the results of his observations of many cases over a period of 12.5 years. He concluded that increased pain is connected with storms, either with or without rain and snow, and also with the lowering barometric pressure. Variations in humidity alone did not seem sufficient to produce or relieve pain, but they may have been effective in conjunction with other factors. He observed further that the situation of the person studied relative to the path of the storm was significant. The intensity of the pain was in proportion to the nearness of the storm and its abruptness of onset. He concluded that pain is aroused by some influence emanating from storms. Just what this influence was he did not know because he could not link it up closely enough with any single atmospheric change, such as a fall in barometric pressure. In 1929 Rentschler, Vanzant and Roundtree[3] published results of their experiments on arthritic pain in relation to changes in weather. They took observations on 367 patients with different forms of arthritis over a period of time not exceeding a year. They found that many of their patients with arthritis, through an increase of their pain, sense the approach or presence of storms. They found as the result of their work that 70 per cent of the time there was a relationship between the curve of pain and barometric pressure curve. For 21 per cent of the time the relation was equally as definite but as one line went up the other went down. In only 7 per cent of the time was a relationship undemonstrable. For 90 per cent of the time there was a relation between the presence of storms and an increase of pain. Observa-

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tions on humidity, temperature, and atmospheric electricity are inconclusive, although they might be effective in certain combinations.

The subject for this experiment was severely wounded in France during the World War and as a result he spent two years in the hospital. The wound consisted of a gunshot wound of the hand and shoulder as a result of which the little and ring fingers were amputated at the metacarpophalangeal articulations and the index and middle fingers at the distal one-half of the second and third metacarpals. In the shoulder there was a loss of the acromion process, the tip of the clavicle, the head of the humerus down to the surgical neck, and a loss of the clavicular and acromial portions of the deltoid muscle. The shaft of the humerus was ankylosed to the scapula at the glenoid fossa. The wound left a scar approximately 2 by 3 inches.

While in the hospital, the subject experienced continual pain, the intensity of which varied to a greater or lesser degree at irregular intervals. At times the pain was very severe and seemed almost unbearable, and this was accompanied by a mental state in which life seemed dark and futile. At other times the pain was easily bearable and life took on a brighter aspect. After the wounds were healed the continual pain disappeared but the subject experienced and still experiences recurrence of pain at irregular intervals. Sometimes the pain feels as though blood were being forced through sore blood vessels. Sometimes it is sharp and lightning-like, while at other times it is in the form of a dull throbbing ache at which time the arm and hand feel hot and tight.

The subject made attempts at predicting the approach of storms or weather changes, using the recurrence of pain as an index. However, he soon found that often the pain appeared without any visible change of weather conditions. As a result of this a record of the recurrence of pain was kept to determine whether there is any relationship between the occurrence of pain and changes in the weather conditions.

PROCEDURE

The procedure consisted in the subject's recording the day and time of day of the recurrence of the pain for a six-month period, extending from January 1 to July 1. The meteorologic conditions for this period were obtained and plotted together so that the relationship, if any, could be easily seen. Data were obtained for barometric pressure, humidity, temperature, wind velocity, and sunshine. Readings for the barometric pressure, humidity, and temperature were taken three times a day, namely at 7 A.M., 2 P.M. and 7 P.M. Readings for wind velocity and sunshine were for the entire day and for only five months, extending from February to July.

RESULTS

The data obtained in this experiment are represented in graphic form in Fig. 1 and in table form in Tables I and II. In Fig. 1 the occurrence of pain is indicated by dashes placed at the points where pain occurred. The point used to determine whether pain occurred on an increase, decrease, or level is where the pain starts. In the six months of observation, pain occurred 50 times. Of these, 3 happened in January, 8 in February, 8 in March, 10 in April, 7 in

May, and 14 in June. The meteorologic agents are first considered separately to determine whether any one alone is responsible for the recurrence of pain.

Temperature.—In the six-month period pain occurred 50 times, 39 of which occurred on a decrease of temperature, giving a correlation of 78 per

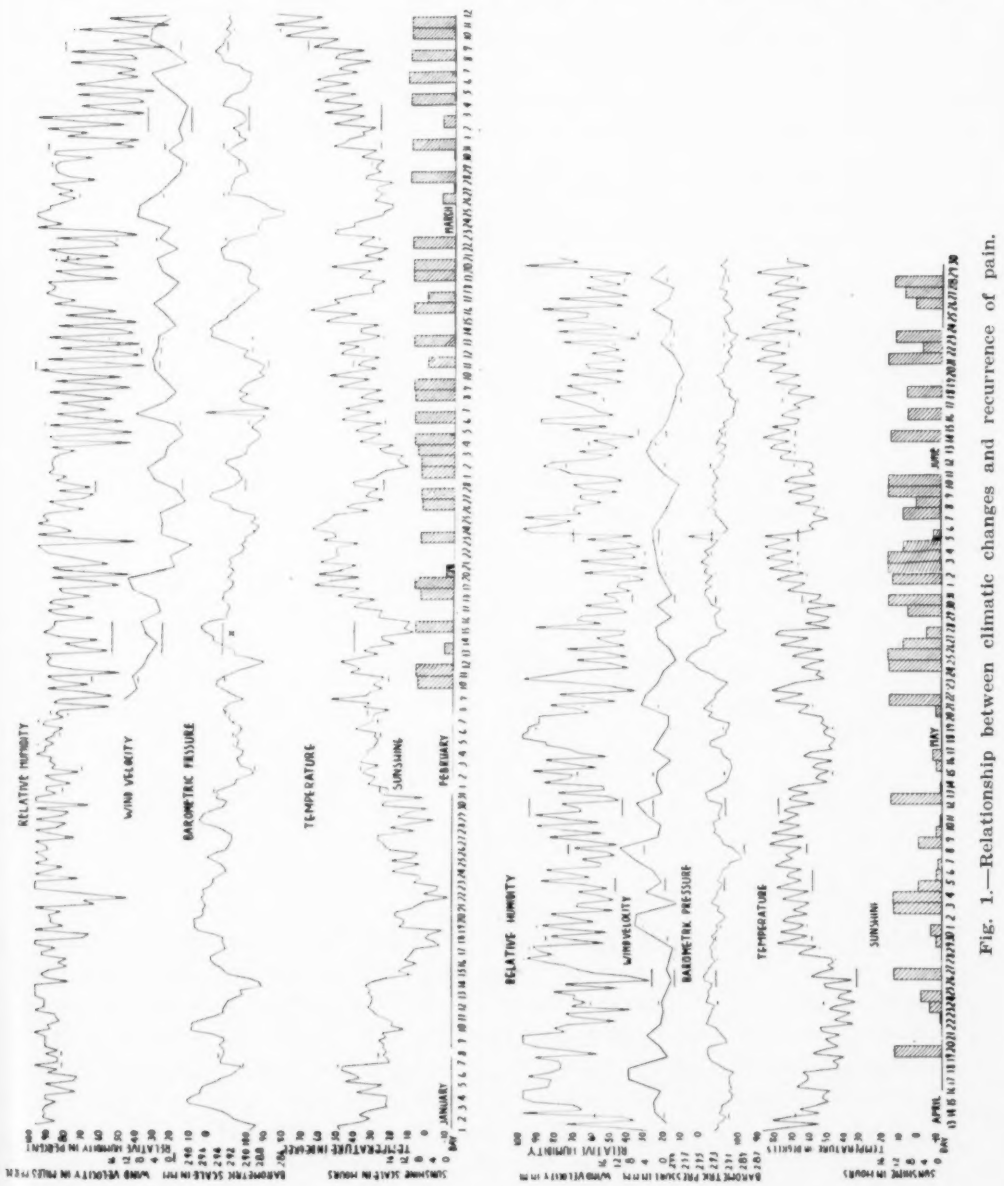

Fig. 1.—Relationship between climatic changes and recurrence of pain.

cent. Ten of the remainder occurred on an increase in temperature giving a correlation of 20 per cent. In one instance pain occurred on the level, a correlation of 2 per cent. During the six months, temperature dropped 172 times and increased the same number of times. Of the 172 times that temperature dropped, pain occurred on only 39 of the times, giving 22.67 per cent. Out of

the 172 times that temperature increased, pain occurred only 10 times, giving 5.8 per cent. The above data mean that of the total number of times that pain occurred 78 per cent occurred on a decrease of temperature, 20 per cent on a raise and 2 per cent on stationary temperature, and of the total number of times that temperature dropped, pain occurred on only 22.67 per cent of them, and of the number of increases pain occurred on only 5.8 per cent of them. The range of temperature at which pain occurred was 23°-92° F. while the temperature range for the six months was –10°-98°. Thus, we see that pain does not occur at the greatest temperature extremes for the six-month period.

Humidity.—Of the 50 times that pain occurred during the six months, 38 of them occurred on an increase of humidity, giving a correlation of 76 per cent. The remaining 12 times occurred on a decrease of humidity, giving a cor-

TABLE I

RELATION OF CHANGES IN BAROMETRIC PRESSURE, TEMPERATURE HUMIDITY, AND WIND VELOCITY TO THE RECURRENCE OF PAIN

	PERCENTAGE OF TIME PAIN OCCURRED ON INCREASE OF	PERCENTAGE OF TIME PAIN OCCURRED ON DECREASE OF	PERCENTAGE OF TIME PAIN OCCURRED ON LEVEL	PERCENTAGE OF WEATHER CHANGES HAVING PAIN, AS COMPARED TO TOTAL NUMBER OF CHANGES	
				INCREASE	DECREASE
Barometric pressure	44.0	56.0		22.22	26.67
Temperature	20.0	78.0	2.0	5.81	22.67
Humidity	76.0	24.0		22.35	6.90
Wind velocity	52.4	47.6		62.86	52.63

TABLE II

RELATION OF DEGREE OF SUNSHINE TO RECURRENCE OF PAIN

AMOUNT OF SUNSHINE	NO. OF TIMES PAIN OCCURRED	PERCENTAGE OF TIME PAIN OCCURRED ON DAYS HAVING	NO. OF DAYS HAVING	PERCENTAGE OF TOTAL NO. OF DAYS ON WHICH PAIN OCCURRED
2.5 or less	19	45.00	74	25.67
2.6-6.0	7	16.60	9	77.77
6.0-9.0	3	7.14	6	50.00
9.0 or more	13	30.95	52	25.00

relation of 24 per cent. During the six months humidity increased 170 times and decreased 173 times. Pain occurred on only 38 of the increases, giving a correlation of 22.35 per cent; consequently of the 170 times that humidity increased, 122 of them were not accompanied by pain. Out of the 173 decreases pain occurred on 12 of them, giving 7 per cent. Consequently 161 of the 173 decreases were painless. The range of humidity at which pain occurred was 29-100 per cent. The range for the six months was 27-100 per cent.

Barometric Pressure.—Of the 50 occurrences of pain, 28 of them occurred on a decrease of barometric pressure, giving a correlation of 56 per cent; 22 of them occurred on an increase in pressure, giving 44 per cent. In the six-month period, barometric pressure increased 99 times and decreased 105 times. Of the 99 increases of barometric pressure, pain occurred on 22 of them, giving 22.22 per cent. Thus the pressure increased 77 times without a recurrence of pain. Of the 105 decreases in barometric pressure, pain occurred on 28 of

them, giving 26.66 per cent. This leaves 77 decreases out of 105 at which pain does not occur. The range at which pain occurred was 28.03-29.74 mm. For the six months the range was 28.805-29.865 mm.

Wind Velocity.—In the five-month period in which data were obtained for wind velocity, pain occurred 42 times. Twenty-two of these occurred on an increase in velocity, giving a percentage of 52.4, while 20 of them occurred on a decrease, giving a percentage of 47.62. Since wind velocity increased 35 times, 13 of these were not accompanied by pain and since it decreased 38 times, 18 were not accompanied by pain. The range of wind velocity in which pain occurred was 19.7-23.0 miles, while for the five months it was 14.7-23. As a result of these data one could say that roughly one-half of the time pain occurs on a decrease in velocity and one-half the time on an increase, but this does not mean that pain occurs 50 per cent of the time that wind velocity increases or decreases.

Sunshine.—For convenience, the amount of sunshine was divided into four groups; namely, days having 2.5 hours or less of sunshine; days having 2.5 to 6 hours of sunshine; days having 6 to 9 hours of sunshine; and days having 9 or more hours of sunshine. There were 74 days having 2.5 or less hours of sunshine, during which pain occurred only 19 times, giving 25.6 per cent. Thus, 61 such days out of the 74 were not accompanied by pain. There were 6 days having 6 to 9 hours of sunshine, 3 of them being accompanied by pain, giving 50 per cent. There were 52 days having 9 or more hours of sunshine during which pain occurred 13 times, giving 25 per cent. There were 39 days not accompanied by pain.

Table I shows the percentage of correlation between the recurrence of pain and temperature, barometric pressure, humidity, and wind velocity. Table II shows the percentage correlation between sunshine and pain.

Examination of the many possible combinations of two or more of the various agents failed to reveal anything significant. The greatest correlation is shown by temperature and humidity. In this case, a simultaneous decrease in temperature and an increase in humidity show a percentage of 66. However, although pain occurred 33 times on such a combination, the combination occurred 150 times, thus 117 times temperature dropped and humidity increased simultaneously without occurrence of pain.

DISCUSSION

The results obtained here do not point to any special agent or agents which might be accused of being the causative factor for the recurrence of pain. Although a decrease in temperature, an increase in humidity, and a simultaneous decrease in temperature and increase in humidity, all show high percentages, this does not seem to be significant, excepting to show that of the total number of occurrences of pain, a high percentage occurs when the weather is under such a condition. It does not, however, point to the fact that the weather condition is the cause of the pain. Take temperature for example, here we have pain occurring 39 out of 50 times on a decrease of temperature, which might suggest the fact that a decrease of temperature is the causative factor of the

recurrence of pain. However, one would think that if a decrease in temperature were the causative factor, pain should occur every time the temperature dropped. But here we have the condition where temperature dropped 133 times without the occurrence of pain. This means one of three things, either (1) that a drop in temperature is not the causative factor; or, (2) a drop in temperature is the causative factor but its effectiveness is counteracted by some other agent or agents; or (3) a drop in temperature is not effective alone, but whenever accompanied by some assisting agent or agents becomes effective. This argument also applies to the other climatic factors which show a high correlation.

However, a close examination of the graph for such factors as suddenness of the change, degree of change, accompanying weather changes, etc., or combination of changes, all fail to reveal the secret of the cause of the recurrence of the pain. It can be seen on the graph that often the pain may start on an increase or decrease of weather change and extend over a period during which the factors fluctuate several times before cessation of pain, as represented by X in Fig. 1.

The data obtained in this experiment have the advantage over those of other workers in that they not only consider each factor separately but also in combination. They not only show that changes in such climatic agents as temperature, humidity, barometric pressure, wind velocity, and amount of sunshine, acting alone, are not the causative agent but also any combination of elements does not account for the recurrence of pain. This then answers negatively the suggestion of other workers that perhaps a combination of elements is the causative factor.

No doubt there is some other agent, or agents, as yet unobserved, which accounts for the recurrence of pain.

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GASTROINTESTINAL MANIFESTATIONS OF HYPERTHYROIDISM*

An Analysis of Eighty Cases of Hyperthyroidism with a Report of Four Cases Masked by Digestive Symptoms

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TYPICAL hyperthyroidism is not difficult to diagnose. The symptomatology of the disease is so protean, however, that there are many cases, some mild, some serious, which challenge diagnostic ability to the utmost. In the *formes frustes* type, in which conspicuous symptoms and signs are lacking, the diagnosis is often overlooked. Such cases are frequently diagnosed as neurocirculatory asthenia as shown in an excellent recent review by Bueerman.[1] That mistaken diagnoses are often made can readily be seen when it is realized that there is no single sign or symptom which is a sine qua non of Graves' disease. Thus, the thyroid often is not enlarged nor is exophthalmos present. Weight loss may be absent. Cases are sometimes seen in which there is a bradycardia. McKinley,[2] Morris,[3] Wohl,[4] and others have reported cases in which even the basal metabolic rate may be within normal limits.

Cases of hyperthyroidism that are monosymptomatic or symptom-poor may focus the attention of the medical examiner on an organ or a group of organs not responsible for the disease. Levine and Sturgis[5] were among the first to report instances of hyperthyroidism masquerading for years as heart disease. The literature is replete with examples of hyperthyroidism erroneously diagnosed as incipient tuberculosis or active low-grade endocarditis. Diabetes is not infrequently mistaken for hyperthyroidism because of glycosuria and diminished glucose tolerance not uncommonly present in the latter. Other diseases which sometimes simulate hyperthyroidism sufficiently to require differential consideration are essential hypertension, focal infection, mild Parkinsonianism, syphilis of the central nervous system, disseminated sclerosis, neuroses of the menopause, and diseases of the gastrointestinal tract.

This paper is limited to a consideration of the gastrointestinal symptoms in Graves' disease. Although digestive disorders in hyperthyroidism are common, statistical analyses of these symptoms are few. For this reason, 80 cases of hyperthyroidism that were studied in the wards of the Mount Sinai Hospital were reviewed from the standpoint of the frequency and character of gastrointestinal symptomatology. The results of this study will be referred to from time to time.

Appetite.—The appetite in hyperthyroidism is sometimes sharply increased and may be enormous or capricious. The fire created by the overactive thyroid, however, is sufficiently intense to burn up the ingested food more rapidly than

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it can be assimilated, so that weight loss rarely can be avoided. The increased appetite of hyperthyroidism is probably an overrated symptom and occurs in a small minority of patients. Of the 80 cases analyzed by us, 8 patients or only 10 per cent were noted as having unusually good appetites, 15 stated their appetites were decreased, and 45 noted no change. The appetites of the remaining 12 were not noted.

Diarrhea and Constipation.—Loose bowels are considered among the classical signs of hyperthyroidism. According to Goldzieher,[6] diarrhea is present in over 30 per cent of cases. In Tinker's[7] series of over 4,000 patients who had undergone thyroid surgery, over 10 per cent had diarrhea. In our own series diarrhea occurred in 16 patients, or 20 per cent. Of these, only 4 had diarrhea which may be called severe. Shirer's[8] study of 42 cases in which diarrhea occurred in 14 per cent closely parallels ours. Hypermotility of the gastrointestinal tract, as evidenced by the head of a barium meal reaching the rectum within three hours, has also been shown by x-ray to occur in many hyperthyroid patients. This accelerated passage of gastrointestinal contents is held responsible in some measure for weight loss, since digestion and absorption, which mainly take place in the small intestines, are interfered with.

What is not so well known is that constipation occurs more often in hyperthyroidism than the diarrhea which is so frequently stressed. In Crile's monograph on hyperthyroidism, no mention is made of constipation. Other textbooks likewise ignore this symptom. Yet in Shirer's[8] series, constipation occurred in 30 per cent, which was twice that of the incidence of diarrhea. Similarly, in our own series, whereas 16 patients suffered from diarrhea, constipation was present in 30 patients. In both series, then, the incidence of constipation approximately was double the incidence of diarrhea. In both series, also, the incidence of normal bowel habits was greater than either the incidence of constipation or diarrhea alone.

Not only is constipation a frequent symptom in hyperthyroidism but occasionally a severe obstipation may be present for several days. Dunlop and Davis[9] reported such a case with an obstipation that lasted nine days and was considered as a case of intestinal obstruction. Packard's[10] case had severe constipation as the chief confusing element in the clinical picture. The mechanism of severe constipation, as explained by Timme,[11] is mediated through the parathyroid glands. In his opinion, parathyroid involvement, an inevitable accompaniment of hyperthyroidism, may produce either intense nausea and abdominal pain, or marked bowel spasticity, because of its influence on calcium metabolism. Smithies[12] believes that hyperthyroidism causes a marked stimulation of the adrenals which, in turn, leads to spasticity of the nonstriated musculature of the splanchnic area, such spasticity producing obstinate constipation and occasionally functional obstruction. It may be relevant at this point to cite in detail the following remarkable case, which exemplifies beautifully a case of hyperthyroidism ushered in by symptoms of intestinal obstruction.

CASE 1.—L. N., a man, aged forty-five, was admitted on the service of Dr. Abraham Trasoff on July 12, 1933, because of vomiting, obstipation and pain in the left lower abdomen. These symptoms first began on July 1 and were relieved by a gastric lavage. On July 3 the vomiting and pain recurred. He took a cathartic which effected an evacuation.

Since then, until his admission (nine days), he had had no stool. He also complained of epigastric distress and sour eructations. His immediate past history was irrelevant, except for the loss of 35 pounds within the last three months.

The patient, on examination, was in acute distress, bitterly complaining of pain and holding his hands over the left lower abdomen. To the examiner's hand the abdomen was surprisingly soft throughout. There was moderate tenderness on deep pressure over the left lower quadrant. No masses were felt. The temperature was 99°, pulse 90, respiration 20 per minute. The teeth were carious, the tonsils diseased. The heart was slightly enlarged to the left and a systolic bruit was audible in the third interspace to the left of the sternum. The blood pressure was 136/70.

In view of the persistent vomiting, obstipation, and abdominal pain, a diagnosis of intestinal obstruction, probably in the sigmoid, was made. Several enemas were given but the return flow was only slightly colored. The sigmoidoscope revealed a spastic rectum and sigmoid colon, the mucous membranes being dry and hyperemic. A barium enema likewise revealed spasticity of the sigmoid and descending colon but no obstruction, the flow of the barium readily passing into the cecum. The barium enema curiously accomplished what the previous enemas failed to do, for, on his return from the Roentgen Ray Department, he passed three copious stools, this after eleven days of absolute obstipation and unsuccessful enemas.

The blood count showed no anemia or leucocytosis. The urea nitrogen was 21 mg. per 100 c.c. of blood, blood sugar 120 mg. per 100 c.c. of blood, CO^2 vol. per cent 59, blood calcium 11.9 mg. per 100 c.c. of blood, the Kolmer and Kahn tests negative. Except for a faint trace of albumin, the urine was normal. A low free acidity was discovered on gastric analysis.

The patient felt fairly comfortable in the main during the next two weeks, although frequently complaining of nausea and slight abdominal discomfort. He required enemas. A recheck on the barium enema was productive of no new findings. Until now our attention had been chiefly focused on the abdomen. The apparently acute but spurious emergency over, a more careful medical survey was made. It was noted that the tachycardia persisted, that the vessels of the neck throbbed, that he had a positive von Graefe sign, as well as a decided tremor of the hands. He was excitable and overanxious about his condition. His deep reflexes were hyperactive and a patellar and ankle clonus were present. Further questioning brought out the fact that he was excessively sensitive to hot weather and unusually comfortable in the winter. These highly suggestive symptoms of hyperthyroidism were confirmed by a basal metabolic rate of +54 (August 3). It was further substantiated by a lowered sugar tolerance curve and a blood cholesterol of 135 mg. per 100 c.c. of blood. Lugol's solution was exhibited, and within three weeks resulted in a marked amelioration of symptoms, especially in the allay of his irritability. The basal metabolic rate was +2 on August 22. Because the initial clinical picture was so dominantly that of intestinal obstruction, some members of the staff demurred at a diagnosis of Graves' disease despite the above findings. It was suggested that the iodine be stopped to see whether the symptoms would return. On cessation of the drug (September 1), the symptoms promptly recurred. Within a week the basal metabolic rate rose to +44 and, significantly, abdominal pain again manifested itself. With the resumption of the Lugol's solution, the basal metabolic rate again fell (+31), although not to its previously low level, a fact which has been frequently noted by other observers.

The patient refused thyroidectomy, and he was referred to the out-patient department for roentgen ray therapy. He felt fairly well for several months. On Feb. 27, 1934 he was readmitted to the wards because of weakness, abdominal pain, and loss of weight. He now had a slightly enlarged thyroid, slight exophthalmos, and a positive Moebius' sign. The basal metabolic rate was +59, the blood cholesterol 140 mg. per 100 c.c. of blood and x-ray of the chest showed no evidence of substernal thyroid. An electrocardiogram showed a left axis deviation and slight inversion of T_1 and T_2. He again refused operation and left the hospital against advice.

Follow-up several months later yielded the information that he was receiving radium treatments over his thyroid gland in a Veteran's hospital.

No comment on this case is necessary except, perhaps, to mention that no case of constipation in hyperthyroidism on record lasted as long as eleven days. The obstipation in the case reported by Dunlop and Davis,[9] mentioned previously, was of nine days' duration, and was considered as a form of thyroid crisis.

In contrast to Case 1, I cite one in which diarrhea was so severe as to temporarily cloud the diagnosis of hyperthyroidism.

CASE 2.—Mrs. A. F., aged sixty, was admitted to the service of Dr. Rubenstone because of diarrhea associated with cramplike pains in the abdomen. The diarrhea was of over two years' duration and severe from the beginning. For the past year daily bowel evacuations numbered between four and six and were occasionally mixed with mucus. Treatment throughout was directed toward an ulcerative colitis. During this time, the patient had lost about fifty pounds in weight. The abdominal symptoms were so prominent after admission to the hospital that they swayed the medical examiner to a diagnosis of colitis. *Trichomonas hominis* was found in the stools, but these flagellates per se are not considered pathogenic. After eight days of observation and negative gastrointestinal studies, an underlying hyperthyroidism was suspected because of a persistent tachycardia, wide pulse pressure, coarse tremors of the tongue and hands, and pulsating carotid vessels. The basal metabolic rate, several times repeated, ranged between +50 and +60. A bilateral, superior thyroid ligation was performed after suitable preparation with resultant marked clinical improvement.

Pain.—Besides diarrhea and constipation, hyperthyroidism may manifest itself in the gastrointestinal tract by some form of abdominal discomfort or actual pain. In our series, fifteen patients, or nearly 20 per cent, complained of pain of considerable severity. The pain may be referred to as an "ache" or "soreness," a "cramp," "burning" or "indigestion." In Verbrycke's[13] series, 16 of 34 patients had general abdominal cramps and pains. Of the 4 cases reported by Hillemand and Mezard,[14] 3 had epigastric pain simulating peptic ulcer and one mimicked the pain of a spastic colon. Crile believes that hyperthyroid patients are prone to get actual ulcers which he calls "kinetic." This opinion received corroboration by Robertson and Hargis[15] who found a high incidence of gastric and duodenal ulcer in autopsied thyrotoxic patients.

Nausea and Vomiting.—Nausea and vomiting are not infrequent, and when they do occur, may be expressions of severe hyperthyroidism. Frequently they shunt the attention primarily to the digestive tract. Vomiting may be persistent enough to deceive even an astute surgeon into performing a dangerous abdominal operation. Hamburger and Lev[16] reported a series of cases in which two patients presented themselves with such persistent vomiting that a diagnosis of gastric malignancy was entertained. In Verbrycke's[13] series, 9 patients had attacks of severe vomiting. In our series, nausea occurred in 12 and vomiting in 9 patients. The incidence of vomiting in Tinker's[7] series was over 10 per cent. Haines[17] thinks that nausea and vomiting may be the principal and sole manifestations of the crises of exophthalmic goiter. It is Lahey's[18] opinion that persistent vomiting, with its concomitant dehydration and emotional upset, is a more certain indicator of an oncoming crisis than is a sudden elevation of the pulse rate. Similarly, the cases of pernicious vomiting recorded by Hamburger and Lev[16] were considered as thyrotoxic crises. These facts suggest that lack of sufficiently obvious cause for vomiting should arouse a suspicion of an exist-

ing hyperthyroidism. Case 3 is a good illustration of an atypical hyperthyroidism in which vomiting was the chief symptom.

CASE 3.—Mrs. A. B., a housewife of sixty years, was admitted to the Mount Sinai Hospital on Dr. Trasoff's service on July 24, 1934, because of gastrointestinal symptoms of four and one-half months' duration. Prior to this time she was apparently well. Her symptoms consisted of epigastric pain which was practically constant and intractable vomiting. During this period, she was hospitalized twice in another institution where a diagnosis of cholecystitis was made clinically and by cholecystography. She weighed 112 pounds, having lost 42 pounds since the inception of her illness. No food, even water or tea, could be tolerated for the past month. Her past medical history had no bearing on her present illness.

On examination sufficient suggestive signs were discovered to suspect that the epigastric pain, vomiting, and loss of weight had been successfully veiling a hyperthyroidism and were not based on cholecystic or intrinsic gastrointestinal disease. Although there were no characteristic eye signs, a stare, slight but unmistakable, was present. There were marked carotid pulsations with a bruit audible over them. The thyroid gland was not palpable. The pulse pressure was high, the blood pressure being 190/70. Corrigan and capillary pulses were elicited. The heart was transversely enlarged and auricular fibrillation was present. Dermatographia was marked. Scattered areas of pigmentation were seen. The skin was moist and hot and weight loss was apparent. In general the patient looked toxic despite a normal temperature.

A basal metabolic reading was +53. The blood cholesterol was lowered to 136 mg. per 100 c.c. After the administration of Lugol's solution for several days, the patient improved strikingly. The vomiting ceased, the excitability and emotionalism diminished, the heart became less turbulent and the bruit over the neck vessels disappeared. The improvement was continuous for several weeks, successive basal metabolic readings being +28 (August 4), +22 (August 11), +18 (August 25). Only the fibrillation continued. The improvement was interrupted by the patient's inadvertent discovery of a contemplated thyroidectomy by which she was deeply perturbed. That her furtive fears, and later, violent objection to surgical intervention were the causation of the next basal metabolic reading being +35 is indubitable. It was thought preferable to substitute roentgen ray therapy at this time. She was discharged with instructions to continue such treatment. She was subsequently operated upon at the University Hosiptal where the removed gland exhibited a diffuse toxic goiter.

Sometimes patients are seen in whom the gastrointestinal symptoms, prominent though they be, may not be the sole symptoms which divert the attention from the underlying hyperthyroid state. Thus there may be a combined gastrointestinal and nervous system syndrome or the digestive symptoms may be complicated by conspicuous cardiovascular phenomena. Such combinations may give rise to complex or bizarre symptomatology which retard speedy diagnosis. The following case, recently seen in the wards, is illustrative.

CASE 4.—J. B., a man of sixty years, was hospitalized on Dr. Rubenstone's service Dec. 31, 1934, because of multiple symptoms. Ten weeks prior to admission he had had la grippe, and since then suffered from headache, dizziness, and cough. He had lost 30 pounds in weight. Palpitation and progressive dyspnea on exertion were of recent onset. In addition he complained of anorexia, an increasing constipation and occasional epigastric pains. Examination disclosed a moderately elevated blood pressure, sclerotic radial vessels, a heart slightly enlarged to the left, with a systolic mitral bruit. He had occasional transient bouts of paroxysmal fibrillation. His chest findings were suggestive of an organized pneumonia, abscess, or an acid-fast lesion, all of which were excluded by an x-ray of the chest which was essentially negative. Epigastric tenderness and rigidity were sufficiently noticeable, coupled with loss of weight, to warrant the suspicion of a gastric malignancy or biliary tract disease. Perhaps the best way to indicate how serious gastrointestinal disease was considered

is to mention the following studies that were ordered: Gastric analysis, cholecystography, gastrointestinal x-ray, barium enema, icterus index. It was only after the lungs and gastrointestinal tract were shown to be free of disease and the cardiovascular system considered as inadequate to explain all the symptoms present that a basal metabolic rate was suggested, two weeks after admission. This was +62. On Jan. 18, 1935 a conference note reads: "Thyroidectomy advised after proper preparation. Aside from tachycardia no stigmata of hyperthyroidism." Surgical consultation on Jan. 19, 1935 reads: "With the exception of the basal metabolic rate there seems to be no evidence of hyperthyroidism. Suggest check on basal metabolic rate." With Lugol's solution and sedatives, there was a diminution in the basal metabolic rate to +25. On February 18, subtotal thyroidectomy was performed. Pathologic report showed a hyperplastic thyroid gland.

COMMENT

We believe that the statistical analysis and the case histories cited above speak eloquently for themselves and are sufficient proof of the importance for being alert with respect to an underlying and unsuspected hyperthyroid disease in cases which present themselves, hypocritically, so to speak, with the ostensible pattern of gastrointestinal disease. We do not concur in the exaggeration of Verbrycke, who says that "the basal metabolic machine has become one of the most valuable parts of the equipment in the diagnosis of diseases of the digestive tract." But the latter ailments do imitate hyperthyroidism with sufficient frequency to compel us to be goiter-conscious at all times. In being so goiter-conscious, hyperthyroidism often will be mistakenly suspected, but also one will be trequently rewarded by uncovering a hitherto concealed thyroid state. The significance of this cannot be underestimated when one considers how few chronic diseases are so eminently amenable to treatment as is hyperthyroidism.

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THE RED BLOOD CELL SEDIMENTATION RATE IN CHRONIC SINUSITIS, CHRONIC TONSILLITIS, AND DENTAL PERIAPICAL INFECTIONS*

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THE significance of the sedimentation rate of the red blood corpuscles is well known today. In the last few years a vast literature has been published not only on the results of the test in different diseases but also on variations in technic. It has been used as an aid in diagnosis and also to follow the progress of disease. This latter use has been applied particularly in judging the activity of tuberculosis and gynecologic conditions. Stainsby and Nicholls[1] have demonstrated that in rheumatoid (infectious) arthritis the change in the erythrocytic sedimentation rate is an effective index of the course of the disease and that the curve of exacerbations and remissions of clinical signs is closely paralleled by the curve of a rising or falling sedimentation rate.

This work of Stainsby and Nicholls might naturally cause one to speculate to what extent the presence or eradication of a focus of infection might influence the results obtained. In other words, might it not be possible that the presence of one or more of the most frequently offending foci that one encounters in patients with rheumatoid arthritis—viz., dental abscesses (periapical), chronically infected tonsils, and chronic sinus infection—is the true cause of a sedimentation rate above normal and might not the decrease of the sedimentation rate to normal that accompanies clinical improvement be due to the removal of such a focus rather than to improvement of the arthritic condition per se? Very little information on the erythrocytic sedimentation rate in such foci is to be found in the literature on the subject.

Cutler[2] in discussing the application of the sedimentation test in general medicine gives a table of "diseases grouped according to sedimentation rates." In this table under the heading "Not Influencing the Sedimentation Rate" he has the classification "Focal infections, such as abscessed teeth, diseased tonsils, and chronic sinusitis." Aside from this statement no supporting evidence is offered. Allard, Ralston, and Ralston[3] investigated the sedimentation rate in 11 patients, 5 women and 6 men between the ages of fifteen and twenty-eight years, in each of whom 2 or more dental periapical rarefactions were demonstrated by x-ray and in whom no other illness could be shown. Each of the 11 patients showed a decreased sedimentation rate after extraction of the involved teeth, as compared with the rate before extraction. However, of these 11 patients, 7 had sedimentation rates that were within the normal range before extraction. Of the 4 individuals who had increased rates prior to extraction, one was later found to have had pulmonary tuberculosis. The authors state that

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some of their private patients with similar granulomas or cysts at the roots of the teeth had normal sedimentation rates before treatment, but they did not include these results in their series. Their chief conclusion from their small number of cases is that "infections at the roots of the teeth may produce a general reaction in the body."

Rault[4] gives as his conclusion that the "sedimentation index is believed to be of value in determining whether pathologic changes are taking place because of pulpless teeth. It is necessary that other sources of infection be eliminated because the sedimentation test is a nonspecific test for infection." In support of this contention he gives his results in a series of 300 cases, in which there was roentgen evidence of infection about the apices of the teeth and in each of which a physical examination had ruled out the presence of other disease. Rault states that in 85 per cent of this series the sedimentation rate was above normal and that after extraction of the involved teeth the sedimentation rate returned to normal. He briefly describes 5 cases in this series as illustrative of his results. However, in only one of these 5 cases was there no evidence of a complicating factor. Of the other 4 patients, one showed a disappearance of certain gastrointestinal symptoms coincident with the fall of the sedimentation rate following extraction, a second had a glaucoma that improved following extraction, another had renal calculi, and in the fourth there was a conjunctivitis that cleared following the removal of the dental focus. Rault also gives a table of 20 additional cases chosen at random. These patients were all men between the ages of nineteen and twenty-five years who had passed the rigid physical examination required for admission to the United States Army. Of these 20 men there were 15 in whom there was no roentgenographic evidence of infection about the roots of the teeth. All of these had sedimentation rates within the normal limits. Of the 5 men showing x-ray evidence of infection about the dental apices, only one had a sedimentation rate above normal. The author seems to have drawn an erroneous deduction from his own facts; contrary to his conclusion, elevation of the sedimentation rate was not the rule in his series of uncomplicated cases of dental periapical rarefaction.

In a group of 102 children with the diagnosis of chronic tonsillitis, Fritsch[5] found that 79 had sedimentation rates that were not above the accepted normal. In each of the 23 cases that had an increased sedimentation rate, he was able to find another condition that would explain the elevation. In those cases where sedimentation rates were checked after the removal of the tonsils, no material change was observed. His conclusion is that chronic hypertrophic tonsillitis does not raise the sedimentation rate.

THE PRESENT STUDY

In order to clarify further this subject, the sedimentation rates were determined in a group of cases in which a diagnosis of chronic tonsillitis, chronic sinus infection, or infection about the dental roots had been made. The patients making up the first two groups were from the Nose and Throat Clinic of the New York Hospital and were seen through the courtesy of Dr. Arthur Palmer. The diagnosis of sinus infection was confirmed in practically every case by x-ray examination and in many of the cases by the character of the sinus washings.

The diagnosis of diseased tonsils was made by members of the Nose and Throat Department after a careful examination of the throat. In the attempt to eliminate from this series patients who might have other ailments, a history was taken and a general physical examination made, but detailed laboratory procedures were not employed for this purpose. The dental cases were made available by Dr. Milton Gabel. The Dental Clinic of the New York Hospital is solely for those patients in whom the dental condition may play a rôle as a complicating or possible etiologic factor in another ailment. Through the cooperation of Dr. John O. McCall of the Guggenheim Dental Clinic, a group of children with infections about their dental roots was examined.

TECHNIC

The technic employed was essentially that described by Rourke and Ernstene[6] in which the sedimentation rate is adjusted according to the volume of the red blood cells. Heparin was used as the anticoagulant. The corrected sedimentation index (C.S.I.) expresses the fall of the red blood cells in millimeters per minute. The upper limit of normal after correction for variation in cell volume was taken as 0.4.

RESULTS

There were 30 cases in which a diagnosis of chronic sinusitis had been made (Table I). Various sinuses and combinations of sinuses were involved. The ages ranged from six years to forty-two years; there were 14 males and 16

TABLE I

ERYTHROCYTE SEDIMENTATION RATE IN CHRONIC SINUSITIS

PATIENT	AGE (YEARS)	SEX	C. S. I.
S. F.	6	M	0.15
S. L.	7	M	0.2
M. B.	8	M	0.1
M. B.	9	F	0.25
A. F.	11	M	0.2
A. L.	11	M	0.15
S. W.	13	M	0.2
F. W.	14	F	0.55
I. K.	14	F	0.4
P. B.	15	M	0.3
M. D.	19	F	0.5
I. M.	19	M	0.4
M. R.	21	F	0.4
A. R.	21	M	0.25
P. W.	21	F	0.3
M. M.	21	F	0.38
K. Y.	22	F	0.2
M. N.	22	F	0.35
B. S.	24	F	0.4
M. M.	25	F	0.2
M. K.	28	F	0.25
M. C.	30	F	0.3
C. B.	34	M	0.55
C. W.	35	F	0.2
M. E.	35	M	0.25
A. S.	35	F	0.6
F. M.	37	M	0.2
W. C.	38	M	0.2
Y. G.	42	F	0.3
F. L.	42	M	0.4

TABLE II

ERYTHROCYTE SEDIMENTATION RATE IN CHRONIC TONSILLITIS

PATIENT	AGE (YEARS)	SEX	C. S. I.
C. K.	5	M	0.2
B. M.	6	M	0.1
W. F.	12	M	0.35
E. K.	19	F	0.4
E. L.	20	F	0.35
L. T.	21	F	0.25
N. M.	22	F	0.3
D. M.	23	F	0.4
G. T.	25	M	0.3
E. C.	25	F	0.2
A. B.	27	F	0.15
O. R.	36	M	0.2
P. C.	38	M	0.3

TABLE III

ERYTHROCYTE SEDIMENTATION RATE IN CHRONIC SINUSITIS AND CHRONIC TONSILLITIS

PATIENT	AGE (YEARS)	SEX	C. S. I.
H. Z.	8	F	0.2
J. E.	23	M	0.1
V. B.	26	M	0.15
W. P.	26	M	0.27
S. T.	67	F	0.4

TABLE IV

ERYTHROCYTE SEDIMENTATION RATE IN DENTAL PERIAPICAL INFECTIONS

PATIENT	AGE (YEARS)	SEX	C. S. I.
J. W.	5	M	0.22
J. S.	6	M	0.1
A. P.	6	F	0.3
C. E.	7	F	0.3
C. M.	7	F	0.3
B. C.	7	F	0.2
C. C.	7	M	0.2
D. O.	7	F	0.1
G. M.	7	M	0.2
H. C.	7	M	0.16
S. H.	8	F	0.19
L. T.	8	M	0.4
G. S.	9	F	0.1
C. R.	9	M	0.4
A. C.	10	M	0.29
S. G.	10	F	0.22
P. C.	10	M	0.38
E. R.	10	F	0.18
L. M.	10	F	0.2
D. D.	10	F	0.3
E. D.	11	F	0.2
A. C.	11	M	0.4
P. P.	14	M	0.2
T. R.	14	M	0.2
L. M.	15	F	0.53
G. H.	23	M	0.2
A. N.	42	F	0.48

females. Twenty-six or 87 per cent of this group had a corrected sedimentation index of 0.4 or less. Of the 4 cases where the corrected sedimentation index was above normal, the highest reading was only 0.6, certainly not very much increased.

In 13 patients ranging in age from five to thirty-eight years, a diagnosis of chronic tonsillitis was made (Table II). There were 6 males and 7 females. In none of these was the corrected sedimentation index above 0.4.

There were 3 males and 2 females with both chronic sinusitis and chronic tonsillitis (Table III). In no case in this group was the corrected sedimentation index over 0.4.

In the dental group (Table IV), the only means of eliminating any complicating factors was by taking a short history and making a physical examination at the time the patient appeared for the extraction. The presence of periapical infection was determined in each case by x-ray or by detection of an abscess after extraction. The blood for the test was taken before removal of the tooth. In some of the individuals there were 2 or more involved teeth. There were 27 patients in this group, 13 males and 14 females. Twenty-five members of the group ranged in age from five to fifteen years. Twenty-five of the 27 patients, or 93 per cent, of this series had a corrected sedimentation index within the normal range. There was but a slight elevation of the corrected sedimentation index in the other 2 patients, the readings being 0.48 and 0.53.

DISCUSSION AND SUMMARY

A determination of the erythrocyte sedimentation rate was made on 30 individuals with chronic sinusitis, 13 with chronic tonsillitis, 5 with both chronic sinusitis and chronic tonsillitis, and 27 with dental periapical infections. There were an equal number of males and females. Of the total of 75 cases, 69, or 92 per cent, had corrected sedimentation indices within normal limits. Of the 6 patients with a corrected sedimentation index above normal, the highest reading was 0.6, and this cannot be considered a marked increase. I believe that if the 6 patients having a corrected sedimentation index above normal had been followed for a longer period of time and had received more detailed laboratory examinations, another condition might have been revealed that would account for the moderate elevation of the corrected sedimentation index.

CONCLUSION

Chronic sinusitis, chronic tonsillitis, or chronic infections about the roots of teeth do not cause an increase above normal values in the sedimentation rate of the red blood cells.

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SUBACUTE BACTERIAL ENDOCARDITIS PRODUCED IN RABBITS WITH STREPTOCOCCI THAT RESEMBLE DIPHTHEROIDS*

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THE authors have in a previous publication[1] described two cases of subacute bacterial endocarditis from which streptococci that resembled diphtheroids were isolated repeatedly from blood cultures during life and in one case from the aortic valve vegetation at autopsy. In the second case autopsy was not done. Considerable emphasis was placed on the finding of these diphtheroid-like organisms, since some investigators have indicated that the finding of diphtheroids in blood cultures is relatively unimportant. The organisms isolated from the above two cases, and similar organisms from other cases of subacute bacterial endocarditis, have been used in the present study to produce, experimentally, subacute bacterial endocarditis in rabbits by the intravenous route. Successful attempts have been made also to reproduce as nearly as possible the clinical picture observed in Case 1 of our previous report.

The organisms used in this study correspond closely with those described by Thomson and Thomson[2] who made a rather thorough study of a hemolytic streptococcus isolated from a case of subacute bacterial endocarditis, which on solid media produced diphtheroid-like forms. Microscopic examination of the vegetations taken from the mitral valve at autopsy of this case showed large numbers of gram-negative organisms of a diphtheroid appearance with polar granules and many cocci. The authors considered this organism to be a hemolytic streptococcus, producing diphtheroid-like involution forms on solid media.

In an extensive review by Thomson and Thomson[2] of experimental endocarditis produced in rabbits, it is quite noticeable that in most instances it was necessary to produce primary damage to the endothelium or valves in order to bring about the desired experimental condition. Rosenbach[3] produced this condition by passing a sound or glass rod down the carotid artery and thus injuring the aortic valve. Orth[4] and Wyssokowitsch[5] injected pulverized carbon simultaneously with streptococci and staphylococci and produced endocarditis in rabbits. They found that intravenous injections alone of streptococci or staphylococci failed to produce endocarditis unless the valves had been previously injured by manipulation as shown by Rosenbach. Ribbert[6] produced endocarditis by injecting intravenously staphylococci simultaneously with an emulsion of

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potato in salt solution thus again effecting a predisposing condition. Prudden[7] accomplished the same end by previously treating the valves with caustics. Bonome[8] observed the development of endocarditis in rabbits by merely injecting intravenously cultures of staphylococci, provided the cocci were in clumps large enough to constitute emboli. Dreschfeld[9] apparently was the first, however, to produce experimentally endocarditis by the simple injection of streptococci into rabbits.

Of particular interest, because of its application to this study, is the work of Haden[10] who published his experimental observations on animals inoculated with streptococci from dental infections, since a streptococcus with a diphtheroid phase was isolated from one of the cases he reported, and the primary source of infection appeared to be an abscessed tooth.

All organisms used in the present study were streptococci of the alpha or beta types which had the ability to develop a diphtheroid-like phase on the proper type of media. On blood agar medium, definite hemolysis occurred of the alpha type, in most instances. The predominating organisms on this medium were streptococci with only occasional pleomorphic, diphtheroid-like forms. On repeated transfer to Loeffler's medium the majority of forms that developed were diphtheroid-like in appearance. In broth cultures the latter forms were not found.

The beta type streptococci were moderately virulent for rabbits on intravenous injection, the alpha type less virulent, whereas in the diphtheroid-like phase these organisms were definitely avirulent in the sense that as much as 10 ml. of a concentrated suspension could be injected intravenously without noticeable effect. A complete study of the bacteriology of these organisms has been previously reported.[1]

EXPERIMENTAL DATA

Three methods were used in an attempt to produce subacute bacterial endocarditis in rabbits.

Casein Method.—Rabbits in this series were injected with emulsions of the streptococcus phase, diphtheroid phase, or mixed phases in 2 per cent casein suspensions. The injections were made intravenously every four to five days in increasing amounts, ranging from 2 to 10 ml. depending upon the virulence of the organism. The total amount injected ranged from 40 to 80 ml.

Four rabbits were injected in this series. Over a period of from eighteen to forty days these animals were killed in turn and autopsied. Except for the one injected with the beta streptococcus phase, at no time did these animals appear sick. Even the animal that was treated for forty days with the diphtheroid phase did not show the marked emaciation observed in rabbits treated by other methods.

Results.—At autopsy no heart damage was found grossly and none could be demonstrated microscopically. In one rabbit there was a question of joint involvement and in another definite kidney involvement was demonstrated. From this short series of experiments, it was evident that the use of casein could not be depended upon to bring about endothelial damage. It seemed advisable

to damage the heart mechanically and therefore all rabbits that were used in later experiments had definite damage of the endothelial tissue brought about by inserting a needle through the heart wall.

Intravenous Method.—In this series, the rabbits were anesthetized and 10 ml. of blood removed from the heart for culture with a 17 gauge, one and one-half inch needle. In all instances these blood cultures were sterile. Before the needle was withdrawn, it was carefully rotated to bring about the necessary damage. The day following the heart puncture each rabbit in this series received an intravenous injection of the phase of the organism under study. Following the first injection, the animals were starved to lower their resistance. The injections were continued every fourth day until death occurred. In this series, 10 rabbits were injected, 3 with the streptococcus phase, 4 with the diphtheroid phase, and 3 with a mixture of both phases. The average time these rabbits were under test was twenty-eight days.

Results.—Of the 3 rabbits injected with the streptococcus phase, 1 showed a gross vegetation on the aortic valve which, when sectioned and properly stained, showed both diphtheroid forms and streptococci, 1 showed a swelling of the cusp, and 1 was negative. Of the 4 rabbits injected with the diphtheroid phase, 1 showed a gross vegetation on the mitral valve, 2 showed swelling of the mitral cusps in each case with a small clot on the ventricular surface, and 1 was negative. The 3 rabbits, injected with the mixed phase, showed no gross vegetations, but all 3 showed thickening of the mitral valve leaflets and 1 of these also had a small tenacious clot.

Tooth Method.—In one of the cases previously reported,[1] the patient, twenty-three years of age, was admitted to the Meriden Hospital on Jan. 31, 1933. He had a previous history of rheumatic fever in 1921. Two weeks prior to admission, the patient had influenza and on entrance to the hospital complained of weakness and fever. A streptococcus with a diphtheroid phase was isolated from the blood on several occasions. X-ray examination showed an abscessed tooth which was extracted. From this abscess an organism was isolated which was shown to be identical morphologically, biochemically, and serologically, with the organism isolated from the blood cultures. The patient died on March 20, 1933, and at autopsy the same organism was found in the large, pale gray, friable vegetation attached to the aortic valve. In view of the above history, the following method was used in an attempt to simulate the clinical picture of previous heart damage caused by rheumatic fever, a lowering of resistance caused by an acute upper respiratory condition, and a focus of infection, an abscessed tooth.

A series of 25 large, white, healthy rabbits were chosen and preliminary blood cultures were made under anesthesia. In all instances these blood cultures were sterile. At the same time the blood cultures were taken, the endothelium of the heart was damaged as previously described. While under anesthesia, the upper, right incisor tooth of each was drilled with an electric drill.* The cavities were opened on the frontal surface of the tooth and then turned, following the root canal as nearly as possible. The cavities were made

*The authors are indebted to Dr. J. J. Roche of Hartford for the dental work done on these rabbits.

sufficiently deep to reach the pulp of the tooth and in some rabbits penetrated the jaw bone. A small, sterile pledget of cotton, saturated with the phase of the

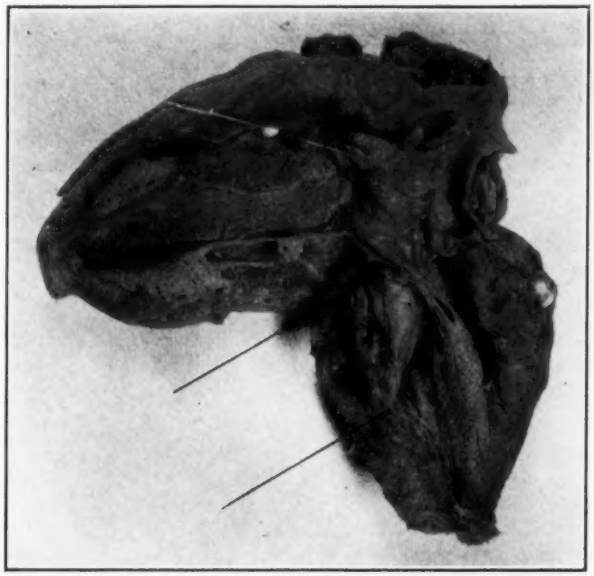

Fig. 1.—Shows large friable vegetation attached to the inferior surface of the mitral valve.

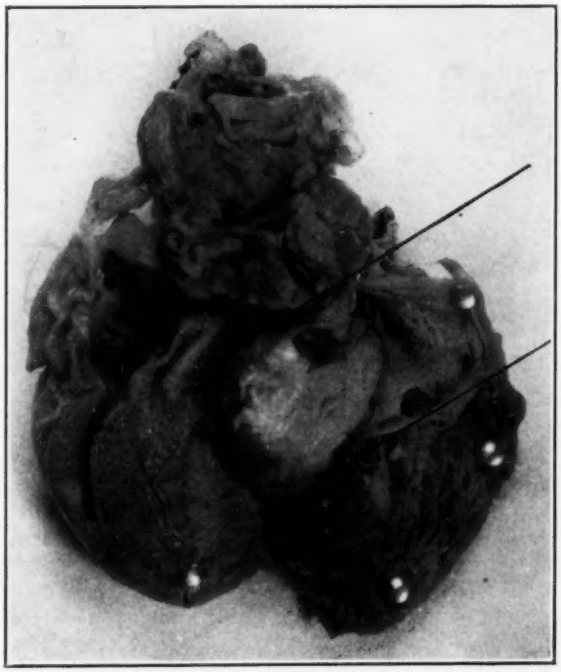

Fig. 2.—Shows large vegetation attached to both the inferior and superior surface of the mitral valve.

streptococcus under study, was forced into the cavity. The remainder of the cavity was then filled with cement. After the focus was established, each rabbit

was given a nose and throat spray with *Haemophilus influenzae*. This treatment was repeated every fourth day. The rabbits were given short rations, and, except for periodic blood cultures and nose and throat spray of *H. influenzae*, the animals were not disturbed again until autopsy. The time from tooth injection to date of autopsy varied between eighteen and sixty-six days.

Results.—Of the 25 rabbits whose teeth were injected in this series, 4 were used for the streptococcus phase. The average time these animals were under test was twenty-eight days. At autopsy 1 of these rabbits had a gross vegetation on the mitral valve, 1 showed thickening of the mitral valve leaflet, 1 showed a pericarditis, and 1 was negative.

Fig. 3.—Shows two rabbit hearts with small but definite whitish vegetations on the superior surface of the mitral valve.

Fig. 4.—Shows low power appearance of the vegetation from the heart shown at the left in Fig. 3. ×70.

The teeth of 18 rabbits were treated with the diphtheroid phase. The organisms used in this series were isolated from 2 cases of subacute bacterial endocarditis. In this group of rabbits, 4 showed gross vegetations on the mitral valve, 11 showed swelling of the mitral valve cusps, the majority with small clots attached, 1 showed a pericarditis, 1 showed questionable valve damage, and 1 was negative. It is interesting to note that the four rabibts showing gross vegetations were injected with the diphtheroid phase of a streptococcus recently isolated from a fatal case of subacute bacterial endocarditis.

The teeth of 3 rabbits were treated with a mixture of both phases; 1 of these showed a verrucous thickening of the leaflet and 2 were negative.

All 25 rabbits in this group had definite abscess formation at the base of the tooth injected, many of which had penetrated the superficial bony structure

of the jaw bone. The periodic nose and throat spray resulted in most instances in an acute coryza with a purulent, mucous discharge from the nose. The blood cultures which were taken at weekly intervals early in these experiments were negative, but progressively in a greater number of instances streptococci were recovered from the blood stream as the upper respiratory condition became established. Forty per cent of the rabbits in this group showed positive blood cultures at some time during the period they were under test.

Summary of Pathologic Observations.—Because of the poor results obtained with the casein method, complete histologic studies were not made of these 4 rabbits. Of the 35 rabbits treated by the intravenous and tooth methods, 7 showed gross vegetations on the heart valves at autopsy. These vegetations were grayish red and friable, all on the left side of the heart, 1 attached to the aortic

Fig. 5.*—Gram stain of section similar to Fig. 4. Shows chains of streptococci and diphtheroid appearing forms in the vegetation. ×1000.

valve and 6 to the mitral valve. Gram-Weigert stains of sections from these valves showed short chains of streptococci and diphtheroid-like forms. These lesions resembled grossly and microscopically the vegetations of subacute bacterial endocarditis that are found in the disease in human beings.

Sixteen rabbits showed lesions on the mitral or aortic valves that did not resemble the typical lesions of subacute bacterial endocarditis. These lesions grossly were about the size of a common pinhead, reddish in color and occurred along the line of closure or slightly nearer the valve base. They would not wash off with running water. Microscopic study of the sections through these foci showed a thickening of the valve leaflet due mostly to a swelling of the stroma. The endothelial layer was swollen and in some instances broken. Over the surface a few red blood cells and a few strands of fibrin were adherent to the

*The authors are greatly indebted to Jean Keiffer of Norwich, Connecticut, for the photographs represented in Figs. 5 and 6.

swollen endothelium. The stroma was infiltrated with about 8 to 10 mononuclear cells, a considerable number of erythrocytes and in some sections occasional polymorphonuclear leucocytes were observed. Gram-Weigert stains of sections from these lesions did not show bacteria. These lesions did not appear to be specific histologically. Twelve rabbits in the series of 35 studied showed no gross nor microscopic lesions on the heart valves.

Although all of the rabbits in the group whose teeth were injected with the diphtheroid phase received the same treatment, certain of these animals showed gross vegetations of typical subacute bacterial endocarditis, others had nonbacterial lesions of a valve, while others were negative. From our observations it is apparent that there is a wide variation in the susceptibility or resistance to infection in a group of rabbits such as these studied. In a previous publication[11] one of us with Mickle demonstrated a similar variation in rabbit

Fig. 6.—Illustrates the appearance of the lesion which we have called prebacterial. The valve is considerably thickened, the stroma is swollen and infiltrated with mononuclear cells and occasional polymorphonuclear cells. The endothelium of the valve is broken and a small amount of fibrinous material may be observed on the endothelial surface. Suitable stains of this section and others in our series show no bacteria. ×100.

resistance to streptococci. We feel that the negative results obtained in some instances and the questionable ones in others, may be accounted for by variability of animal resistance.

DISCUSSION

In the present study, it was possible to produce subacute bacterial endocarditis in rabbits by two methods. It is apparent from these experiments and those of others that primary damage to the endothelium of the heart is essential to the successful development of the disease. Further, because of the particularly low virulence of the strains used in this investigation, it was necessary to drastically lower the resistance of the rabbits under test, and either to inject massive doses of these strains or to establish a well-defined focus of infection.

The gross vegetations resembling the heart lesion of subacute bacterial endocarditis in human beings, found in seven rabbits, or 20 per cent, need no further comment, since they were equivalent to the typical endocardial lesions found in this disease. However, the lesions observed in 16 rabbits, or 45.7 per

cent, that did not show bacteria in suitably stained sections, require some consideration. A careful study of these lesions indicates that they represent, though nonspecific histologically, the initial stage in the development of subacute bacterial endocarditis. The failure of these lesions to develop into the bacterial phase appears to us to be due to the commonly observed wide variation in animal resistance. The correct interpretation of experimental lesions produced in rabbits offers considerable difficulty. However, 20 per cent of the hearts in our series showed gross vegetations, unquestionably of the subacute bacterial endocarditis type, in which streptococcal and diphtheroid-like forms were observed. Since all animals were similarly treated by one of the two successful methods used and since 34.3 per cent showed no demonstrable heart lesions, it would appear logical to assume that the lesions described as prebacterial and found in 45.7 per cent of our series were due to the organisms with which the animals were injected. In that sense these lesions may be considered specific.

There is a resemblance between the "prebacterial" valve lesion in rabbits which we have described and the bacteria-free lesion of the endocardium in rheumatic endocarditis. Publications are accumulating which suggest the probable etiologic rôle of the streptococcus in this disease. Cecil, Nichols and Stainsby[12] have found streptococci in blood cultures in 83.9 per cent of a series of 31 cases. Callow[13] has demonstrated that diplostreptococci (alpha type) and pleomorphic bacilli may be recovered repeatedly from the blood of patients with rheumatic fever. Thomson and Hill[14] report a certain type of streptococcus present in the throats of rheumatic fever patients constantly. They do not yet claim that it has any real etiologic significance, but regard it with strong suspicion because of its persistent frequency. Early in his studies of specific therapy in rheumatic fever, Small[15] came to the conclusion that a particular group of streptococci was the etiologic agent in this disease.

It is generally accepted that no bacteria are found in the lesion of rheumatic endocarditis, and there is little cellular exudate. This lesion is considered specific for rheumatic endocarditis, and it is undoubtedly a result of the action, perhaps a sensitization, of the causative agent of rheumatic fever.

Freiberg and Dorst[16] are of the opinion that in certain types of arthritis the lesions found are not due to the active infection in the joints themselves, but are rather an expression of sensitized tissue to an antigen which remains in a persisting focus elsewhere in the body. Von Glahn and Pappenheimer[17] recently stated in their article on the relationship between rheumatic and subacute bacterial endocarditis that their studies lead to the conclusion, "it is not the old scarred valve that is predisposed to subsequent infection with bacteria, but the valve or auricular wall which bears on its surface the fresh unhealed verruca or plaque."

The prebacterial lesion described in the present studies resembles the lesion found in rheumatic endocarditis and appears to be due to the streptococcus with which the rabbits were injected. Whether the early lesion is a sensitization to an antigen which has an affinity for the heart is questionable, but it does seem probable that this injury to the valve endothelium prepares it for later

implantation of bacteria when the body defenses of the test animals are drastically impaired. To obtain this implantation on the heart valves of rabbits, it is essential that organisms of low-grade virulence be used. Under the experimental conditions produced in our animals, virulent organisms would have caused death, probably by septicemia, in so short a time that subacute bacterial endocarditis could not have developed. Apparently the criterion of low-grade virulence is fulfilled when the streptococci are in the diphtheroid phase. These studies would indicate that the organism is in this phase when it becomes implanted on the heart valves.

We have been impressed by the striking frequency of colds and influenza which precede by two or three weeks the beginning manifestations of subacute bacterial endocarditis. This point seemed important, since it was felt that possibly the acute infection interferes with the normal defense mechanism of the endothelial system, thus lowering the resistance of the body to such an extent that a focus or foci of low-grade streptococci, easily held in check under normal conditions, were allowed to filter into the blood stream. If the valve endothelium or mural endocardium had been prepared or sensitized and had the prebacterial lesions as described, then the conditions would probably be favorable for the implantation of organisms of low-grade virulence which normally would have been easily taken care of by the body defenses. Following this premise we sprayed the upper air passages of a number of our rabbits with *H. influenzae* and subjected them to partial starvation. In this way we endeavored to duplicate the conditions which we observed to exist immediately preceding the development of subacute bacterial endocarditis in human beings. The foci of low-grade organisms experimentally produced in the rabbits were held localized until the acute upper respiratory infection had become established. At this time the organisms, previously localized, flooded the blood stream of those animals whose resistance had been sufficiently lowered. This was shown to be true by periodic blood cultures. It would appear that during these showers of bacteria, organisms became attached to the lesion, which is described in this paper as "prebacterial lesion" and from this time on the development of the typical vegetation of subacute bacterial endocarditis progressed.

SUMMARY

Subacute bacterial endocarditis may be produced in rabbits by endocardial damage, followed by the intravenous injection of massive doses of streptococci of low-grade virulence; or, by establishing a focus of these streptococci in the teeth after previous endocardial damage, followed by drastic interference with the resistance of the animal.

Valvular lesions were produced in 65.7 per cent of our series. Twenty per cent showed gross vegetations which were typical of subacute bacterial endocarditis and 45.7 per cent showed an early injury to the valve which we have termed the "prebacterial lesion."

Evidence is presented which appears to show that the early or "prebacterial" lesion is the first stage in the development of the vegetation of subacute bacterial endocarditis.

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THE LEUCOPENIC INDEX IN VASOMOTOR RHINITIS

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THE leucopenic index as appraised in several highly consistent accounts of recent experience gives added weight to its importance as a new diagnostic method in the practice of allergy. The first communication by Vaughan[1] in 1934 reported its use based upon the observation that in the leucocyte response to foods, a rise in the white cell count follows the ingestion of compatible foods, but if sensitizing or incompatible foods are ingested, there is a corresponding and generally consistent depression of the white blood count. The leucopenic index facilitates a more rapid diagnosis of allergy as well as increased accuracy in segregation of the suspected allergens for clinical test or other type of investigation. Gay[2] has made a report on the leucopenic index with respect to the relation of allergy and peptic ulcer. The work of Rinkel[3] dealing with a rather wide range of sensitizations, exhaustively and over a long period of time, indicates greater precision and better results from pretreatment routines and subsequent management of patients because of the method. His interpretation of the various types of leucocytic curves and the factors which modify the response has enhanced its clinical application. Vaughan[4] finds a 5.2 per cent increase in diagnostic efficiency on the part of the leucopenic index over other earlier methods of investigating allergic conditions. My own experience, based upon reported work[5] with twelve patients with intractable asthma, shows an increased efficiency of 21.7 per cent over skin tests and other usual clinical routines.

Our test conditions have been made as nearly identical as possible in every case. We consider it important to use the same pipette for every reading in a series, with thorough agitation of the cells in the pipette, and ample time allowance for settling of the cells in the counting chamber for accurate readings. Such conditions as the emotional state of the patient, previous physical exertion, preparation and quantity of the test food, uniform temperature of the room, and absolute rest during the test, all may influence the counts and must receive consideration. Detailed attention to these factors will result in white blood counts with not more than 5 per cent error. The patient reports in the fasting state and is kept at absolute rest throughout the period during which the counts are made. After an initial rest period of thirty minutes, a white blood count, known as the fasting count, is made. The test food is then ingested and white blood counts are done at fifteen to twenty-minute intervals until the series of readings adequately reflects the trend of increase or decrease in the counts.

The weight of the leucopenic index hinges upon the proper interpretation of the plotted curves of the serial white blood counts. Rinkel and Gay[6] have described the various types of curves which may be obtained. In general, it may be stated that a compatible food, not productive of symptoms, reveals an increasing elevation of the serial white blood count beyond the forty-minute period,

after which the elevation may increase still further, or it may then fall into a gradual decline. The incompatible or symptom-producing food is represented by several types of curves, the most common of which reveals a gradual depression of serial white blood counts beyond the forty-minute period. The curve presenting wide fluctuations varying between increases or decreases from the fasting base should be regarded as an incompatible type, as studies have shown that the food producing this curve frequently results in a definite incompatible character when repeated subsequently. Rinkel and Gay[6] emphasize the importance of the stationary curve at the forty-minute period, followed by leucocytosis as representative of an incompatible type of curve. Indeterminate curve responses reveal little or no fluctuation from the fasting base. It has been found that the indeterminate and widely fluctuant curve may be more definitely interpreted when the food in question is tested again at a later date.

Any impression this statement of procedures may give that the method is offered as a formula for diagnosis is soon dispelled by the consideration of any single series of leucocytic readings. Clinical evidence must be adduced in every instance to corroborate the curve in question. Consideration must be given to changing and degrees of food sensitizations which will result in variations of leucocytic responses accordingly. Rinkel[3] emphasizes the leucocytic transitions from positive to negative, or the reverse, in relation to the incidence of the test food. Food diaries may show an unaccountably delayed response of two weeks or more to known food sensitizations even though the food is ingested daily throughout this period, or the allergic reaction may not occur until the incompatible food is repeated several times in the diet within several days. These factors should receive close consideration in the interpretation of correlating clinical symptoms and the leucocytic responses to a food.

In the author's intractable asthma series mentioned earlier, readings showed positive findings in the leucopenic index with negative skin reactions on the same substances and somatic signs failed to establish the foods in question in 8.5 per cent of cases. The leucopenic index was corroborated by positive skin tests in 2.83 per cent of patients who developed allergic symptoms after ingestion of the questioned foods. In 11.32 per cent of the intractable asthmas the ingested foods produced symptoms in the presence of a leucocytosis and skin tests were negative.

However, clinical symptoms were produced by only 1.9 per cent of the foods which resulted in a leucocytosis in the presence of positive skin tests, while 34.9 per cent of foods resulting in negative skin tests with a leucopenia proved to be allergic under clinical tests. The method proved more accurate than the usual skin tests in diagnosis, although the demonstration of a leucopenia with or without a correspondingly positive skin test does not establish the fact of sensitization or exclude it without clinical corroboration.

The present study includes a consideration of leucopenic index determinations in 23 patients affected with vasomotor rhinitis. In 105 determinations there are 46 instances of leucopenia, four of which were negative when tested clinically and by means of skin tests. There were 13 positive leucopenic readings fully corroborated by skin tests and clinical symptoms. In 28 instances leucopenia with clinical symptoms exhibited negative skin tests. Summarized, this repre-

sents 26.67 per cent of total tests, ignoring the four cases of leucopenia without clinical symptoms and one case not brought to clinical tests, with full corroboration. This is a diagnostic value of the leucopenic index over skin tests of 21.91 per cent.

There were eleven foods clinically negative, but positive on skin test and without leucopenia, while four clinically negative foods, negative to skin tests, showed a leucopenia. One food productive of symptoms showed a positive skin test without the leucopenia. One other food was positive clinically with negative skin test and no leucopenia. This emphasized our earlier statement that the blood picture may not correspond with clinical symptoms and the leucopenic index is not to be accepted as absolutely specific, but its increased diagnostic

TABLE I

ANALYSIS OF 105 LEUCOPENIC INDEX COUNTS IN 23 CASES OF VASOMOTOR RHINITIS

CASE	DURATION YEARS	ALLERGENS TESTED	BLOOD COUNTS		SKIN TESTS		CLINICAL RESULTS		
			LEUCOPENIA	LEUCOCYTOSIS	POSITIVE	NEGATIVE	GOOD	FAIR	POOR
KKJ	1½	3	2	1	1	2			1
BT	7	9	4	5	1	8	1		
CO	3	7	5	2	1	6	1		
FJ	2½	4	1	3	2	2	1		
JJ	4	3	3		1	2		1	
TH	5½	3	1	2	2	1		1	
DLG	1½	3	1	2		3	1		
HE	12	2		2	1	1			1
MB	1½	3	1	2	1	2		1	
OB	½	3				3		1	
JB	50	10	3	7	6	4	1		
RG	9	4	3	1		4	1		
VK	6	8	2	6		8		1	
VN	4	6	2	4	1	5	1		
AP	3	4	1	3	1	3			1
CH	25	3	2	1		3	1		
MC	20	3	1	2		3	1		
JJ	4	7	5	2	3	4		1	
VV	5	3	2	1	1	2	1		
TLor	4½	4		4	2	2	1		
HH	6	4	2	2	1	3		1	
TLz	24	3	2	1	1	2	1		
EOB	1	6	3	3		6		1	
Totals		105	46	59	26	79	12	8	3

TABLE II

ANALYSIS OF 105 LEUCOPENIC INDEX COUNTS IN 23 CASES OF VASOMOTOR RHINITIS

	BLOOD COUNTS		CLINICAL TESTS			SKIN TESTS		PERCENTAGE
	LEUCOPENIA	LEUCOCYTOSIS	POSITIVE	NEGATIVE	NOT TRIED	POSITIVE	NEGATIVE	
		45		45			45	42.86
		11		11		11		10.47
	4			4			4	3.81
		1	1			1		0.95
		1	1				1	0.95
	13		13			13		12.39
			28				28	26.67
	28	1			1	1		0.95
	1				1		1	0.95
Totals	46	59	43	60	2	26	79	
Totals	105		105			105		100.00

accuracy seems clearly demonstrated. Many cases of vasomotor rhinitis are refractory to allergic management, and because of negative skin tests, failure to respond to elimination diets, vaccines, glandular therapy, and other therapeutic procedures, are frequently classed as being nonallergic in character. It is this type of case in which the leucopenic index is particularly valuable. Rigid dietary regimes are often imposed on these individuals, selecting foods which are considered least likely to produce symptoms. And yet, the very foods which have been selected have often been demonstrated as contributory in the etiology of the condition. The leucopenic index will often establish such foods as lettuce, coffee, spinach, celery, rice, asparagus, apples, peaches, and other fruits and vegetables, as a cause of symptoms, and previously not given due consideration as such because of the limitations of the usual diagnostic procedures. Not only can a more specific diet be prescribed in a much shorter period of time, but the diet can often be planned to include some of the staple foods, such as wheat, eggs, milk, and potatoes, all or most of which are excluded from the usual trial diets imposing inconveniences and hardships to the patient.

With respect to inhalants, these findings would seem to apply as well to airborne allergens as to ingested agents. One patient, known to be sensitive to dog hair, complained of a "scratchy" feeling in the throat within ten minutes after exposure to this specific agent. This was followed by the development of itching and congestion of the nasal mucosa. Meanwhile the white blood count dropped 500 cells at the ten-minute period, and the leucocyte count fell to 1,400 cells below the fasting count within twenty minutes from the time of the initial exposure. At the end of an hour the leucocytes again approached the fasting count, the symptoms having subsided as the normal white blood cell count was regained.

Sensitization, even in the absence of clinical symptoms, cannot be ruled out when the allergen in question results in a markedly lowered white blood count. An increase in the test dosage may result in clinical symptoms which corroborate the blood findings. Degrees of sensitization may vary in the same patient from week to week and such changes are reflected in the blood picture as the sensitizations change. Differential blood counts were undertaken in a number of these leucopenic observations with the result that, at times, an increasing eosinophilia was seen to occur as the white blood count decreased, but the observation was so infrequent that its diagnostic import did not seem to warrant the procedure.

CONCLUSIONS

From the data in hand, it appears that the diagnostic accuracy of the leucopenic index materially exceeds the usual skin tests and clinical routines. It is of particular value when intractable conditions show few or no positive skin tests and fail to respond to usual allergic management. The superiority of the procedure over the usual diagnostic methods more than compensates for the time and painstaking effort involved. The leucopenic index facilitates more rapid and accurate diagnosis leading to improved specific therapeutic measures and results in vasomotor rhinitis.

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THE LEUCOPENIC INDEX AS A DIAGNOSTIC METHOD IN THE STUDY OF FOOD ALLERGY

With a Discussion of Its Reliability

Warren T. Vaughan, M.D., Richmond, Va.

THE leucopenic index was developed in 1933 by the writer[1] as an outgrowth of an observation he had made some years previously, that although the Widal colloidoclastic reaction was not a reliable test for liver function, certain persons allergic to milk reacted with leucopenia after the ingestion of milk during the test. This suggested the possibility that it could be used for the demonstration of milk allergy rather than as a liver test. Also that, if the test should prove specific, it could be used with other foods to demonstrate the presence or absence of allergy.

This was found to be true in a sufficiently high proportion of cases to justify the development of a method for clinical use. The ingestion of a food to which a person is allergic will usually produce a leucopenic response, with a drop in total white count of more than 1,000 cells per c.mm. A food to which one is not allergic usually produces no such drop, may indeed be followed by a rise.

The technic of the leucopenic index is briefly as follows: Two fasting white counts are made at ten-minute intervals. The patient then eats the food for which he is to be tested. Following this, three additional counts are made at half-hour intervals. If any of the postprandial counts falls more than 1,000 below the mean of the two fasting counts the index is considered positive. A drop of less than 1,000 is considered negative unless it is maintained throughout the three counts, in which case it is described as a "general low range, probably positive." The same pipette and counting chamber are used throughout each study. Pipettes are shaken three minutes to ensure even distribution. Counting chambers are filled evenly and to approximately the same degree each time. At least 200 cells are counted (eight 1 mm. squares) in each determination. Blood is obtained from a freely flowing needle puncture.

Applying this method clinically we found in a series of 191 tests that 118 or 62 per cent showed a correspondence between the skin reaction and the

leucopenic index.[2] If the skin reaction was positive, the leucopenic was also positive and vice versa. In 21 the leucopenic index was positive although the skin reactions had been negative. Ten of this latter group found that the substances causing leucopenia also caused allergic symptoms. In these the index was the only means by which we were able to identify offending foods.

This first series represented patients with unknown sensitizations in which the index was used with the skin test, to determine etiologic factors. A second series[3] was then run with patients whose idiosynerasies were known prior to the application of the test. Twenty-six patients, known and proved to be allergic to stated foods, were tested both by the leucopenic index and the skin test. Twenty-one reacted positively to the offending food by the index, 17 by skin test. There was 81 per cent correspondence between food allergy and the leucocytic response as contrasted with 65 per cent with the results of skin testing.

Forty-three control tests with the same foods, in non-sensitive individuals gave but six positive leucopenic indices. One of these, a positive reaction to tomato, occurred in an individual who had undoubtedly previously been sensitive to tomato although he had ceased some time previously to experience symptoms therefrom. The same may have been true in some of the other false positive reactions.

In this second series the test was carried out for a total period of four hours. The most pronounced drop sometimes did not occur until three and one-half or four hours after ingestion. For the simplification of the test, it was found that if it be run for but an hour and a half, about 8 per cent of positive reactions would be missed. In using the index one should, therefore, bear in mind that there may be delayed positives which do not show up within the arbitrary test period. Therefore, in case of doubt this period should be prolonged to two, three, or even four hours.

It was shown that the leucocytic response to wheat, egg, and milk was at a lower level in allergics than in nonallergics. That is, there was as much fluctuation up and down from highest to lowest counts, but the total range for allergics occurred in a lower band of total white counts than the nonallergics. This is probably equally true for other foods. The general upward trend of the nonsensitive response tends to confirm the conception of a low grade normal digestive leucocytosis in some persons.

The usefulness of the leucopenic index has been confirmed by Rinkel[4] who has brought out the fact that intractable asthmatics sometimes give positive reactions to nearly all foods; by Zeller[5] who emphasizes its value in intractable asthma; by Gay[6] who recommends its use in the study of peptic ulcer; and by Squier[7] who suggests that additional information can be obtained if total eosinophile counts are done synchronously with total white counts.

The present communication deals particularly with an intensive investigation of a single patient who is so unfortunate as to be allergic to a very large number of foods and who, fortunately for the study, responds symptomatically very promptly and in a clear-cut manner to offending foods.

Her symptoms include migraine, urticaria, angioneurotic edema and colonic pain.

She was tested by the standard technic with 31 foods. Sixteen failed to cause symptoms. With one exception none of these foods was followed by a leucocyte drop of 1,000 or more (Chart 1).

Fifteen foods caused symptoms. Every one of these was followed by a drop of more than 1,000 at some point in the postprandial curve (Chart 2). The severity of resultant symptoms is indicated on the chart in degrees of 1-plus, 2-plus, etc. With one exception (grape) the severity of the reaction corresponded quite closely to the degree of depression of the white count.

Charts 3, 4, and 5 show the total range of the curves in response to foods causing no symptoms, mild symptoms and severe symptoms. The more pronounced depression, accompanying more severe reactions, is evident. The response to honeydew in Chart 4 is of especial interest. At the end of ninety

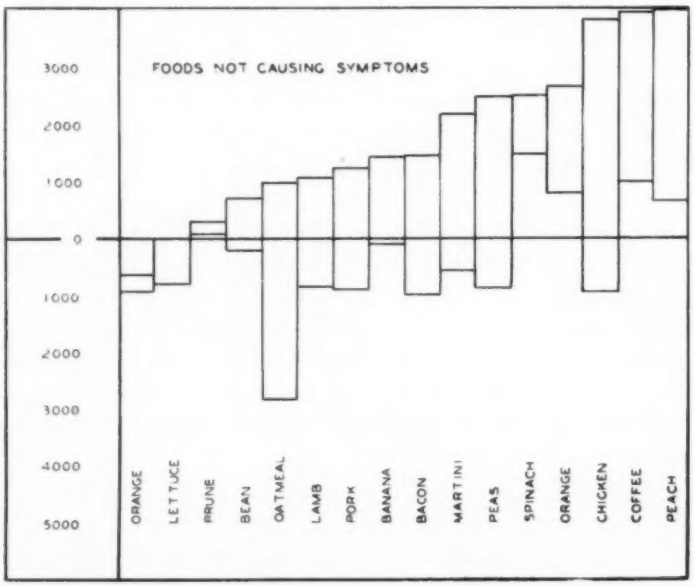

Chart 1.

minutes (the standard test time) the index was still negative but the patient had already responded with migraine. An additional count was therefore done at the end of two and one-half hours, at which time the index had become positive. This was a delayed positive reaction, a false negative index with the test as routinely applied. It emphasizes the need for continuing the count in case of doubt.

The curves of response to pork are of interest (Chart 6). Obviously, ham, bacon, pork, and pork sausage are entirely distinct foods, but, irrespective of other ingredients and methods of preparation, they all do contain pork. With the exception of ham there is a general similarity to the curves, all of them being of the "Z" type, suggesting the possibility of a consistent curve of response to a given food.

The most interesting feature of the pork curve is the observation that pork at one time produced a positive index, *with accompanying symptoms,* while

at another time the index was negative and *there were no symptoms.* We may speak of the patient in the latter case as being sensitive to pork but in allergic equilibrium at the time of the test. These two types of response to pork in

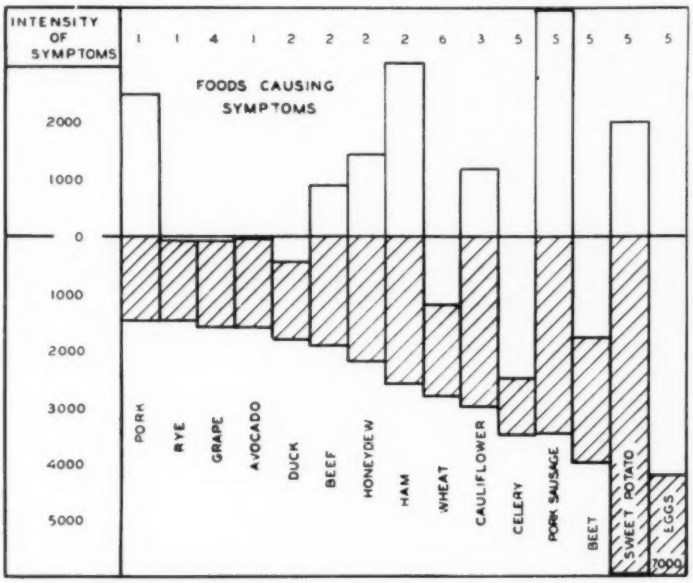

Chart 2.

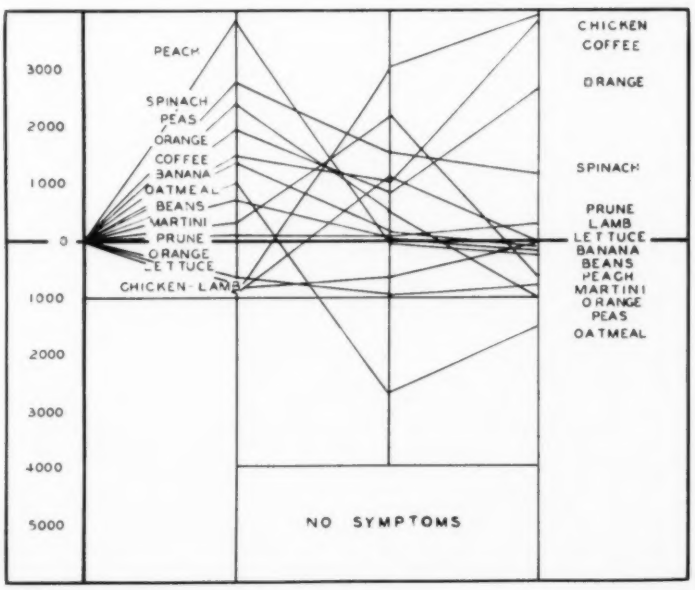

Chart 3.

the same patient justify the designation of an 800 drop or thereabouts as "borderline" and as indicating that the patient may actually be allergic to this food. In this event repetition of the test would be in order.

DISCUSSION

If the leucopenic index is to establish itself as a diagnostic measure useful in food allergy, it must fulfill certain criteria. It must be based upon estab-

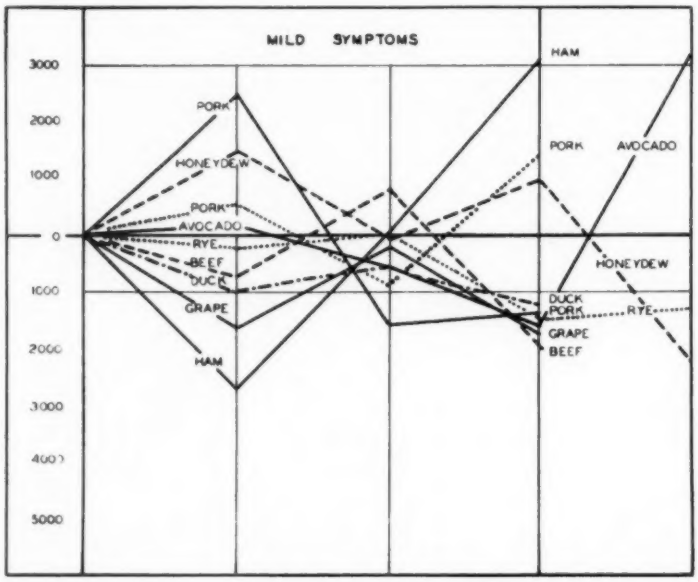

Chart 4.

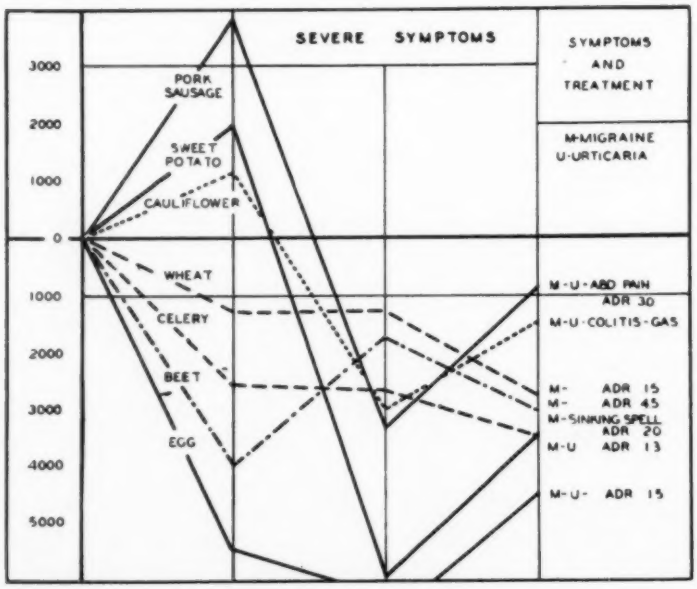

Chart 5.

lished facts. It must be the measure of a response which is the rule rather than the exception. It must have a reasonably high degree of accuracy. It must be made as simple as possible consistent with reasonable accuracy.

Of the occurrence of digestive leucopenia following the ingestion of an allergenic food there can be no doubt. Leucopenia forms part of the picture of experimental anaphylactic shock. My own observations and those of the authors above mentioned demonstrate a clinical correlation. I have recently been fortunate in obtaining Joltrain's monograph[8] on the "Hemoclastic Crisis in Urticaria" which contains numerous, sometimes startling illustrations of leucopenic response to the ingestion of a specific allergen. In it Joltrain states that Widal's original work on the hemoclastic or colloidoclastic crisis dealt with it as an allergenic response. It was not until later that he suggested its use as a test for liver function.

The question of the most desirable procedure for the routine test may still be an open one. In our clinic we are using the routine and criteria which

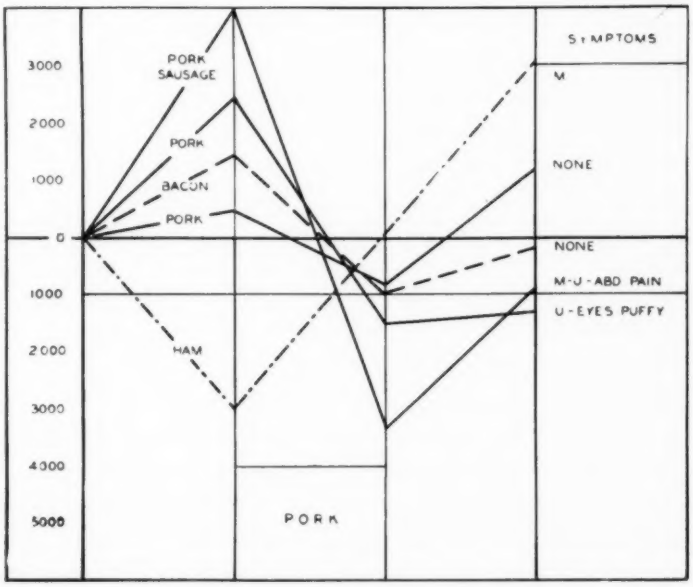

Chart 6.

I first suggested in 1934, except that instead of taking the mean of the two fasting counts as a base line, we now take whichever of the two counts is the lower and require a drop of 1,000 cells or more below this lowest fasting count.

A drop of 1,000 is an arbitrary requirement but is based upon the sound fact that in the vast majority of instances the fluctuation of total white counts in a fasting individual is usually not over 2,000. The upward fluctuation of 1,000 is ignored, the downward fluctuation of 1,000 is adopted as a standard. Occasionally one sees much wider fasting fluctuations. In one case we observed a fluctuation, within ten minutes, of over 3,000. A number of factors enter into this, some of which are not controllable. The total number of leucocytes in one capillary bed may be quite different from that in an adjoining bed at the same instant. However it has been shown that simultaneous counts taken from different fingers are the same, within the limits of normal error. Accuracy of laboratory technique including method of puncture, amount of squeezing,

care in filling the pipette, consistent delivery of the same volume of fluid into the counting chamber, and accuracy in the actual counting are all factors that can be controlled up to a certain limit. An even distribution is *most important* and requires hand shaking of the pipette for at least three minutes. Extreme accuracy throughout the procedure is indispensable.

Rinkel, likewise Gay, find that a drop of but 500 cells may be significant. Since this fluctuation may be exceeded in the fasting state and since it is scarcely outside the limits of error in white counting, I feel that a 1,000 drop is a safer criterion. Joltrain believes that a drop of 2,000 cells or more is indicative of a shock reaction. He, however, has dealt with explosive cases with pronounced allergic symptoms, reactions that correspond more to those illustrated in Chart 5, above. A mild but definitely positive reaction which we so often observe would be overlooked if we adopted the criteria of Joltrain. For the present therefore I feel that the original standards of interpretation should be retained, except as mentioned, regarding the fasting level. A drop of between 500 and 1,000 may be designated "suggestive" or "borderline"; one of 1,000-2,000 as positive; over 2,000 as strongly positive.

Is the test sufficiently reliable to be utilized effectively? The answer to this question involves a recapitulation of interpretive factors. Too wide a fasting fluctuation may obscure the findings. I have gained the impression that the higher the fasting white count the more likely is there to be a wider fluctuation, although this does not always occur. Also, the higher the white count the more pronounced will be the drop in a positive response. This raises a question as to whether the requisite drop should vary depending upon the original fasting count. Thus, a drop of 1,000 might be required if the fasting count is 6,000 while a drop of 2,000 would be required if the fasting count is 12,000 or 14,000. On this I have as yet no final opinion.

We have seen patients who have reacted positively one day, and who on the next morning still showed leucopenia (around 3,000). We have interpreted these as still showing a delayed positive index. Since the patient is still reacting, one would not expect a further drop, following the ingestion of another allergenic food. This might also occur following the eating of an allergenic food, even though no test was being done. A fasting leucopenia therefore decreases the reliability of the test.

The delayed positive reaction will be missed with the routine procedure. The examiner should be conscious of this fact and be ready to prolong or repeat the test in case of doubt and especially if the final count suggests a beginning drop. Since 92 per cent of positive indices will be so within ninety minutes this justifies simplification, but at the same time completion of the test at ninety minutes diminishes the accuracy of the findings.

Foods which actually cause trouble may at times give borderline and negative indices. This is illustrated in the pork chart (Chart 6). One should realize this and repeat the test where indicated. It is of especial interest to note that in the case described, when the test was negative the patient did not respond with symptoms.

The test may occasionally be positive to a substance which does not cause trouble. In one case (tomato) this was interpreted as a residual effect of a known previous sensitization.

We have run repeated tests with the same food in quite a number of individuals. In roughly 50 per cent the index remained consistently the same, either positive or negative, but in 50 per cent there was a change from positive to negative or vice versa. At first I thought that this indicated an inherent weakness in the test but after further study I have concluded that the factor of variation was more likely to be in the individual, as in the pork test, particularly in view of the fact that these repetitions were done on only very mildly sensitive individuals. It is well known that persons mildly sensitive to certain foods often may eat these foods with impunity just as frequently as they eat them with consequent symptoms. Very highly sensitive persons with pronounced drops tend more to give similar responses each time.

The leucopenic index is not proposed as a substitute for other methods of allergic study. No one procedure can be counted upon to solve the intricate problems of the objective diagnosis of food allergy. The index, with an accuracy of 81 per cent when used as a routine procedure or 93 per cent when performed with utmost care as in the case herein detailed, compares very favorably with the skin test to foods, with its reliability of 65 per cent. However, it possesses the disadvantage that it is most time consuming. We have found it to be a very helpful adjunct to the skin test, using it routinely with a few staple foods, and with other selected foods concerning which there may still be special question.

ADDENDUM

The reliability of the leucopenic index has recently been questioned[9] on the basis of published reports on the great variability of the fasting leucocyte count. The reports of eight investigators or groups of investigators are marshalled to demonstrate that there is such a wide normal variation in total leucocyte counts that conclusions drawn therefrom are open to serious doubt. An analysis of these eight studies is as follows.

It is quite true that there are wide fluctuations in the leucocyte count, both fasting and postprandial, but these occur in the afternoon rather than in the morning. Leucopenic index studies are done in the morning, the patient reporting to the office in the fasting state at 9 o'clock. The study requires about two hours and we usually make only one per day on each patient.

Sabin and her collaborators[10] report all day studies on six persons. Their total ranges in white count from 9 to 11 A.M. in these six series are as follows: 2,000; 1,700; 1,800; 2,500; 2,500; 3,700. Taking the interval 9 to 12 A.M. we find a wider variation as follows: 2,500; 3,000; 3,000; 3,000; 3,500; 6,000. The range through morning and afternoon is much wider, as follows: 6,000; 4,700; 5,200; 6,000; 4,300; 5,000.

Medlar[11] has observed a variation of 50 per cent in less than half an hour. But in his serial counts which were made from 9 to 10 A.M. in two cases and 9 to 11 in one, only six out of a total of 59 counts showed a fluctuation of more than 2,000. Furthermore, since he was making his counts at three- and five-

minute intervals, he was forced to use several pipettes instead of a single pipette throughout the study. It is true that they were Bureau of Standard pipettes but even these have some variation.

Jones and his collaborators[12] found that with counts at fifteen-minute intervals, taken from 9 A.M. to 2 P.M. the counts did not vary over 2,000 in any of the nine cases. This was also true of one additional case run from 9 A.M. to 1 P.M. Only in one case of their series of eleven did they observe a variation of more than 2,000 throughout the whole morning.

Shaw[13] who, like Sabin and her coworkers, found wide variations in the afternoon, observed the following variations in a series of thirteen studies made at fifteen-minute intervals in the morning up to 11 A.M.; 2,100; 2,000; 1,700; 700; 600; 800; 800; 400; 3,000; 1,800; 500; 900; 1,400. In one case studied from nine to twelve there was a variation of 3,000. In thirteen cases run through the morning until 1 P.M. the following variations were observed: 2,800; 1,400; 2,300; 2,000; 1,900; 1,300; 1,400; 500; 3,000; 2,200; 1,800; 1,500; and 600.

The two charts of Mauriac and Cabouat[14] cannot be applied to the discussion since each of them represents only the maxima and minima of half-hour counts throughout the day, on four and five days, respectively. Some maxima occurred on one day while others occurred on other days. There is no way of determining from the charts the actual counts for any one day. Even so, however, in one of these charts it is readily seen that in a period from 10 A.M. to 2 P.M. the variation was less than 2,100, not only in any one day but in all five days put together. The other chart showed a greater variation. These two charts also showed that the range in variation is much greater in the afternoon than in the morning. Their third chart shows a range of 3,000 in successive counts at fifteen-minute intervals over a period of two hours and fifteen minutes, but they do not state whether this counting was done in the morning or the afternoon.

Ponder, Saslow and Schweizer[15] have undoubtedly made the most careful investigations of variations in the white count through the day. In each of their determinations they have counted 800 cells. They find that it is necessary to total this many to eliminate all possible errors in counting. From their careful counts they conclude that the large fluctuations reported by the other authors mentioned above "seem to be due principally to errors of method. The total white cell count shows, however, small fluctuation, not exceeding plus or minus 8 per cent throughout the day."

In one series which they use as an example, counting 800 cells each time, they find that with counts at twenty-minute intervals for three hours and twenty minutes the total variation does not exceed 1,000, that is, 500 up or down. In this same series when only 200 cells were counted 35 out of 44 counts showed a variation of not over 2,000, and only two of the 44 counts represented a variation of as much as 3,200. They do not state whether these counts were made in the morning or afternoon.

In one protocol in which the time of day is indicated as the morning and in which the determinations were made with a count of 200 cells, the greatest variation in the fifteen-minute counts up to noon was 2,080 cells.

These authors emphasize that the chief source of error is faulty distribution of the cells on the counting chamber. They circumvent this by counting large numbers of cells. I have emphasized elsewhere the extreme importance of proper mixing and distribution of the cells. This has always been a most important part of our practice of the test.

Simpson[16] made counts on himself at intervals of five minutes for a total of thirty minutes. He did this four times. In three out of the four times the total range was well under 2,000. On the fourth curve two out of the six counts showed a range of more than 2,000, the total range being 4,000. In other words in a total of 24 counts only two showed a range beyond 2,000. Repeating this on another subject he observed a much wider fluctuation. In one half-hour period the fluctuation was less than 2,000; in two other periods it was between 2,000 and 3,000 and in the fourth period it was over 4,000. All of the counts in both series were done in the morning.

Five series of counts were taken at intervals of one or two minutes. Three stayed within the 2,000 range while two exceeded this, ranging around 3,000. Obviously the possibility of pipette error must be considered in this series as well as in Medlar's series since different pipettes had to be used.

Washburn[17] finds hourly fluctuations of well over 2,000 in healthy infants. This is true in the morning as well as in the afternoon. All infants were under six months of age. Since he finds the total fluctuation of leucocyte counts in normal healthy infants to range anywhere from 5,000 to 20,000 one must be inclined to conclude that the white counts of infants under six months vary more than they do in healthy adults. This is already known to be the case with the newborn.

We have ourselves analyzed a total of 1,000 serial fasting white blood counts made on 500 persons. Each person had two fasting white blood counts made at an interval of ten minutes. All counts were made between 9 and 10 A.M. Among these 500 cases (1,000 counts) only 1 per cent showed a 10-minute fasting fluctuation of over 2,000.

The selection of 2,000 as the normal fasting range is entirely arbitrary, based upon our observation of the range as it occurs in the majority of cases. It might be that 3,000 or even 4,000 would be a safer figure. We might be tempted to change it, to require a drop of 1,500 or 2,000 instead of the 1,000 that is now required, were it not for the fact that, from the wealth of experience that we have had with the index, we have found a drop of 1,000 to be the best criterion, and I know that if this were increased materially we would fail to recognize many allergenic foods which we now discover by this method and which are subsequently proved beyond doubt to be causative of trouble.

The development of the leucopenic index and its simplification as a clinical test procedure naturally went through several evolutionary phases. During this period of study, counts were made every fifteen minutes and the period of study, postingestive, was carried as long as four hours. Differential counts, bleeding and clotting time, and blood pressure were determined simultaneously. However, a test of this sort, in order to be clinically applicable must be simplified as much as possible. Our present procedure represents, I believe, as much simplification as can safely be accomplished. There is necessarily some sacrifice in

accuracy with this simplification. I have found that we lose about 8 per cent in accuracy. The index, as used routinely, is therefore not 100 per cent accurate or reliable but even so it is about 20 per cent more reliable than the skin test to foods (scratch, intracutaneous, or both).

I feel justified in concluding that the bulk of the evidence reviewed warrants the statement that our interpretation of the leucopenic index, as it is done, in the morning hours, is consistent with the available literature on variations of the normal white count.

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The author wishes to express his appreciation of the interest and collaboration of Mr. and Mrs. Ray A. Van Clief of Esmont, Virginia, who have made possible the study of the leucopenic index as it has been developed to date.

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LABORATORY METHODS

THE LABORATORY DIAGNOSIS OF BLASTOMYCOSIS*

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RECENT reports in the literature prove conclusively that blastomycosis (American type) is a disease which is far more common and more widespread than is generally realized. The clinical picture of generalized infection with *Blastomyces dermatitidis* is frequently so similar to that presented by tuberculosis that the clinician is often compelled to turn to the laboratory to establish a diagnosis. Many of the cases reported as blastomycosis have been diagnosed solely upon the finding of the fungus in sections of organs or in sputum, pus, or skin scrapings from the infected patient. Isolation of the fungus from such material is frequently unsuccessful. Benham[1] has called attention to the fact that there are at least 10 fungi capable of causing "systemic infections with granulomatous lesions and which appear in the infected tissue as rounded cells more or less resembling yeasts." Although the fungus causing Gilchrist's disease has certain definite morphologic characteristics in lesions which are practically diagnostic in the hands of one experienced in their identification, it is obvious that confirmation by culture should be obtained whenever possible.

The isolation and identification of this fungus, a relatively simple procedure for the mycologist, frequently present some difficulties to the bacteriologist who is not accustomed to working with fungi and who does not have access at all times to the special media required for myocologic study.

We have succeeded in culturing the causative organism from 9 cases of human blastomycosis and from a dog with spontaneous pulmonary blastomycosis. The only media found necessary for the isolation of the fungus were blood agar plates pH 7.4 and Sabouraud's dextrose agar slants.

The bacteriologic procedures which are of definite value in establishing the diagnosis of infection with *Blastomyces dermatitidis* are:

1. Direct microscopic examination of the unstained sputum, exudate, or scrapings.
2. Isolation and identification of the infecting organism.
3. Skin tests with killed organisms.
4. Complement-fixation tests.

PROCEDURES

Direct Microscopic Examination.—The material is first examined in the usual manner by mixing some of the sputum, pus, or scrapings with 10 to 20

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per cent fresh sodium hydroxide or potassium hydroxide. The organisms are seen as double contoured round or oval budding forms usually 8 to 10 μ in diameter although they may be much larger. Frequently there may be difficulty in differentiating certain artefacts from true blastomycetes. In these cases it is often advisable to make fresh wet preparations without sodium hydroxide, rim the coverslip with vaseline and allow the slide to set at room temperature for twenty-four to forty-eight hours. Blastomycetes in pus and sputum

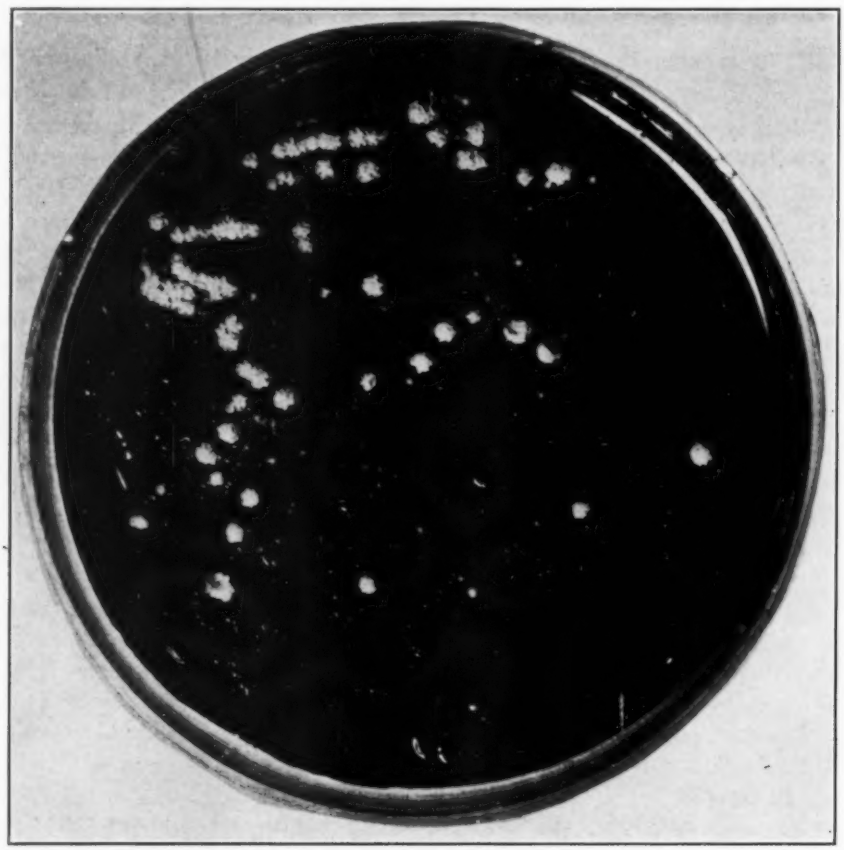


Fig. 1.—Photograph of colonies of *Blastomyces dermatitidis* on blood agar incubated at 37°C. for two weeks.

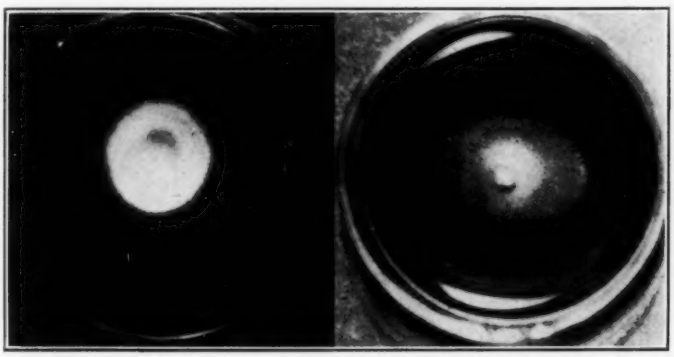
under these conditions frequently germinate to form well-developed branched mycelia.

Isolation of the Fungus.—Cultures of the fungus from the early cases were obtained by planting the infected material on Sabouraud's dextrose agar. Certain difficulties were encountered which led us to the adoption of the following method for routine isolation of *Blastomyces dermatitidis*. The specimens are planted by heavy inoculation on two or more blood agar plates and on several Sabouraud's dextrose agar slants. The slants and some of the blood agar plates are left at room temperature. The remaining plates are incubated at 37° C. All plates and slants are kept at least four weeks before discarding, since it

occasionally takes this long for colonies to develop. Colonies are usually apparent from one to two weeks after inoculation. Media which appear hopelessly contaminated with bacteria or yeastlike organisms should not be discarded, for the blastomycetes are often capable of growing and forming recognizable colonies in spite of the numerous other organisms present.

On blood agar incubated at 37° C., the colonies are small and waxy and do not differ greatly in appearance from colonies of tubercle bacilli (Fig. 1). Aerial hyphae do not appear unless the cultures become very old or dry. The

Fig. 2.—Photograph showing yeastlike cells obtained from one of the colonies shown in Fig. 1. A drop of tincture of iodine was added to the fresh preparation.



A. *B.*

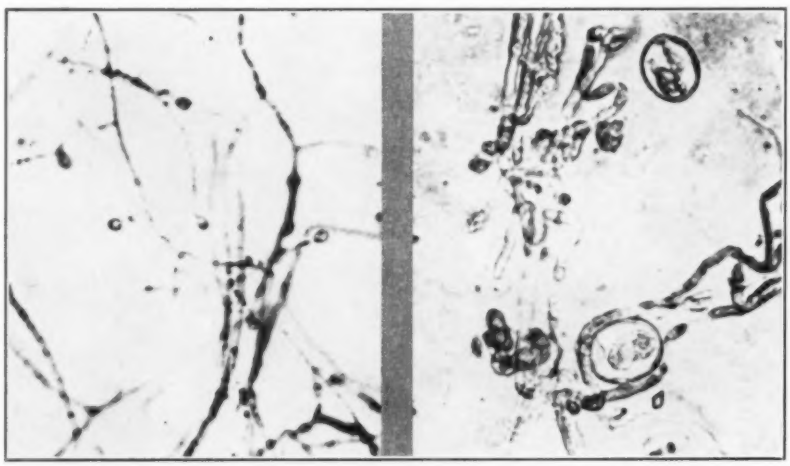
Fig. 3.—*A*, Photograph of a colony of *Blastomyces dermatitidis* on Sabouraud's dextrose agar incubated at room temperature for eight days. *B*, Photograph of a colony of *Blastomyces dermatitidis* on blood agar incubated at room temperature for eight days.

colonies are easy to pick up with an inoculating loop as there are no mycelia holding them to the medium. Smears of this material show numerous budding yeastlike structures with occasional elongated forms resembling rudimentary mycelial elements (Fig. 2).

On Sabouraud's dextrose slants, the organism appears first as a white mold with an abundant aerial mycelium (Fig. 3, *a*). Microscopic examination shows numerous branched hyphae with laterial conidia and thick swollen structures, resembling chlamydospores. Raquette hyphae such as are found in cultures of dermatophytes may be seen (Fig. 4, *a*).

On blood agar at room temperature, the fungus forms a large colony which is characterized by the formation of a mycelium in the medium. The surface growth is small and slightly elevated. Radiating in an orderly manner from this central "button" are the densely packed mycelial strands (Fig. 3, *b*). The colony is so firmly appressed to the medium that it cannot be picked up easily with a bacteriologic loop. On microscopic examination, the colony is seen to be made up chiefly of densely packed intertwined and curled hyphae. Conidia are rare, but some double contoured bodies as well as many large swollen thick-walled chlamydospores are present (Fig. 4, *b*). If the culture is allowed to develop further, an aerial mycelium is formed which may either be soft and fluffy in appearance or the projecting hyphae may grow as if twisted together in coarse coremiform structures.

The yeastlike form of the organism can be maintained by subculturing the organisms on blood agar. After good growth has occurred, the slants are



A. *B.*

Fig. 4.—*A*, Photograph showing the microscopic appearance of a fresh lactophenol preparation obtained from the colony seen in Fig. 3 *A* stained with 0.5 per cent cotton blue. *B*, Photograph showing the microscopic appearance of a fresh preparation from the colony seen in Fig. 3 *B* stained with tincture of iodine.

placed in the ice box until the time of next transplant. If the cultures are removed from the incubator and left at room temperature, aerial hyphae appear within several days. One strain has been maintained in the yeastlike form for four and one-half years. Cultures kept in the moldlike stage by repeated transplants on Sabouraud's medium will develop into waxy yeastlike colonies if subcultured on the blood agar medium. Typical colonies on blood agar were obtained in this way from two strains which had been kept moldlike for fourteen months.

Skin Tests.—We have used a heat-killed suspension of blastomycetes as skin testing material. The vaccine is prepared by suspending some of the yeastlike organisms from the waxy growth on blood agar in sterile saline. The vaccine is diluted until the resulting suspension is faintly cloudy and is then heated to 60° C. for four hours. A sample of vaccine is planted on blood

agar and incubated at 37° C. for at least ten days to insure sterility. The vaccine is preserved with 0.35 per cent tricresol. Skin tests are done by injecting 0.1 c.c. of this suspension intracutaneously. Usually an area of erythema appears about the site of injection within fifteen to twenty minutes. This reaction, however, is not specific, since we have found it in patients with other types of pulmonary infection. The characteristic reaction begins after twelve to twenty-four hours and reaches its maximum in two or four days, resembling very closely a positive tuberculin reaction. Occasionally the reaction progresses to sterile abscess formation.

Complement-Fixation Tests.—A complement-fixation test specific for *Blastomyces dermatitidis* has recently been described by one of us.[2] The antigen for the test is prepared from the fungus grown for two weeks on blood agar at 37° C. The organisms from 2 or 3 slants are washed off in 3 or 4 c.c. of normal saline and ground in a mortar to break up the larger clumps. The anticomplementary titer of the suspension is determined as follows: Normal saline is added in such a way as to obtain dilutions of the original suspension representing 100 per cent, 90 per cent, 80 per cent, etc., to 10 per cent. The quantity of each suspension to be tested should be determined by the customary quantities used in the routine Wassermann test in the laboratory where the test is to be performed. In this laboratory the procedure is to add 0.2 c.c. quantities of each dilution to 0.2 c.c. of saline and 0.2 c.c. complement. This mixture is placed in the ice box for three hours. The rack is then warmed for thirty minutes in the 37° C. water-bath. Sensitized cells (0.4 c.c.) are added, and after thirty minutes' incubation, the degree of hemolysis in each tube is determined.

The suspension used in the actual test is one-third of the greatest dilution which shows the least anticomplementary properties. The patient's serum after inactivation at 56° C. for twenty minutes is diluted 1-4 with saline and 0.2 c.c. quantities added to the same amounts of complement and properly diluted antigen. Fixation is carried out at ice box temperature for three hours, and the rest of the procedure is the same as used in determining the AC titer of the antigen. The results of the test are recorded as 1+, 2+, 3+, and 4+ depending upon the degree of inhibition of hemolysis. The serum and antigen should be controlled for anticomplementary properties by using double quantities (0.4 c.c.) of each reagent.

Minor difficulties and variations in the laboratory diagnosis in individual cases are best illustrated by reporting our experiences with the following patients:

CASE 1.—M. M. was admitted to Duke Hospital with a diagnosis of metastatic carcinoma of the lung. Direct examination of the sputum showed occasional yeastlike bodies, some of which were double contoured. On Sabouraud's medium a moderately heavy growth of monilia appeared several days after inoculation. However, after two weeks' incubation, typical colonies of *Blastomyces dermatitidis* appeared and the organisms were obtained in pure culture by the usual plating methods. No skin tests or complement-fixation tests were done. The patient died and at necropsy the blastomycetes were found by direct examination of lung tissue.

CASE 2.—W. G. Bloody pus was obtained from fluctuating abscesses on the hands and feet. No double contoured organisms were seen on direct examination, but pure cultures of

the fungus were obtained on both Sabouraud's dextrose agar and blood agar. Subculture to Sabouraud's medium failed to grow until laked blood was added to the medium. Subsequent transplants were successful without the addition of blood. Colonies on the original blood agar plate were subcultured on blood agar slants and have maintained their waxy growth and yeastlike form on this medium for a period of four and one-half years. Transplants were made every two weeks during the first year, and the interval between transplants has been gradually lengthened until now they are subcultured every two or three months.

No skin tests or serologic studies were done. The necropsy findings in this case were those of extreme generalized blastomycosis involving practically every organ in the body. Blastomycetes were seen in sections of all lesions.

CASE 3.—R. B. This patient was admitted to a sanitorium with a diagnosis of pulmonary tuberculosis complicated by a pleural effusion. The sputum was negative for tubercle bacilli by direct examination and guinea pig inoculation. Specimens of sputum were planted on Sabouraud's medium and incubated at 37° C. A heavy growth of monilia appeared four days after inoculation and the typical moldlike growth of blastomyces appeared twelve days after inoculation. No skin tests or complement-fixation tests were done.

CASE 4.—S. D. Pure cultures of the fungus were easily obtained by planting pus from the chest wall on blood agar and Sabouraud's medium. Sputum cultures revealed recognizable colonies which appeared only after two weeks' incubation. Skin tests were positive. Repeated serologic tests over a period of seven months showed uniformly positive fixation of complement with his own organism as antigen as well as with antigens prepared from 2 strains of blastomyces isolated from other patients. The patient died and extensive blastomycosis was found at necropsy.

CASE 5.—E. L. The first specimens of sputum were overgrown by aspergilli. Blastomycetes, however, were recovered in pure culture on blood agar and Sabouraud's media inoculated with pus from a subcutaneous abscess on the thigh. Treatment with potassium iodide was started and after several weeks the aspergilli disappeared suddenly from the sputum and blastomycetes were recovered on both types of media. Skin tests were positive as were the complement-fixation tests with two different strains of blastomyces as antigen. The patient died but necropsy was not permitted.

CASE 6.—G. J. This was a case of extensive skin blastomycosis in which numerous budding forms were seen on section and on direct examination of scrapings from the skin. On first admission to the hospital, scrapings were planted on Sabouraud's medium and blood agar but growth was not obtained since the plates were discarded because of the extreme degree of bacterial contamination. On the second admission positive cultures were obtained only on the blood agar plates which were allowed to set at room temperature for three weeks. Because of the very heavy growth of bacteria, pure cultures could be obtained only by clipping the most superficial hyphae with sterile finger nail scissors and transplanting the clippings to fresh media. Skin tests were negative. Complement-fixation tests with one sample of serum were negative with 5 strains of blastomyces. The fungus recovered from this patient fixed complement when mixed with the serum from Case 8.

CASE 7.—A. Y. This patient was admitted with six large fluctuating subcutaneous abscesses. Double contoured organisms were seen on direct examination of pus from one of these abscesses. Because of certain points in the history, one of the attending physicians suspected that this was a case of infection with cryptococci and not Gilchrist's disease. A fresh preparation allowed to set overnight revealed that the organisms produced definite branching mycelia in the pus. This observation ruled out the possibility of cryptococcus infection (Torulosis). Pus planted on the routine media yielded pure cultures on all media. Skin tests were markedly positive and resulted in the formation of a small sterile abscess at the site of injection. Repeated complement-fixation tests were negative with 5 strains of blastomyces, including the strain isolated from the patient. The blastomycete recovered from this patient fixed complement with serums from Cases 8 and 9.

CASE 8.—V. S. This patient was admitted with the clinical diagnosis of primary carcinoma of the lungs. A specimen of material obtained at bronchoscopy showed typical blastomycetes by direct examination and growth was obtained on all the routine media. This patient later had a severe blastomycotic infection of the uterus and peritoneum. Cultures of the pelvic peritoneal organs as well as the peritoneal pus were positive on all routine media. It is interesting to note that typical colonies appeared more rapidly on the blood agar plates kept at room temperature. A skin test was markedly positive with subsequent formation of a small sterile abscess. Repeated complement-fixation tests over a period of fifteen months have been strongly positive with seven strains of blastomycetes including the strain isolated from the patient's sputum.

CASE 9.—R. H. Inoculations of sputum, urine and pus from subcutaneous abscesses by the routine method yielded blastomyces in all instances. Skin tests were questionable. Complement-fixation tests were positive with 6 strains of blastomyces. Necropsy of this patient showed blastomycotic lesions in the lungs, subcutaneous tissues and prostate.

CASE 10.—Dog with spontaneous pulmonary blastomycosis. Smears of the lung made at postmortem showed typical double contoured budding forms. Cultures of the lung on Sabouraud's medium resulted in numerous colonies of monilia resembling those described by Benham[3] as *Monilia krusei*. After several weeks aerial hyphae appeared and pure cultures were finally obtained by the clipping method described under Case 5. No serologic studies were made of this organism.

SUMMARY AND CONCLUSIONS

The laboratory procedures of value in the diagnosis of blastomycosis are discussed and may be summarized as follows:

The usual method of direct examination of sputum or pus may be supplemented by keeping a fresh specimen of the material at room temperature for twenty-four to forty-eight hours. The formation of a branched mycelium proves that a suspected structure is a fungus.

Sabouraud's dextrose agar is valuable as an isolation medium, but cultures should be planted also on blood agar since some strains apparently require blood in the medium for primary isolation.

Specimens apparently hopelessly contaminated with yeasts or bacteria frequently will yield colonies of blastomycetes if the cultures are kept for three to four weeks.

Pure cultures of the fungus often can be freed of contaminating organisms by clipping the aerial hyphae with sterile finger nail scissors and transplanting the clippings to suitable media.

Descriptions and photographs of the macroscopic and microscopic appearances of the fungus when grown on blood agar and Sabouraud's medium are presented.

The yeastlike form of the organism can be maintained on blood agar for as long as four and one-half years and frequently can be recovered from the moldlike type of growth when subcultured on blood agar. This reversion has been accomplished in two strains which were maintained in the moldlike stage for fourteen months.

A positive tuberculin-like skin test with a heat-killed vaccine prepared from the yeastlike form of the organism is obtained in some cases of blastomycosis.

Complement-fixing antibodies are present in the serums of a large percentage of patients with generalized blastomycosis.

It has been demonstrated by the complement-fixation reaction that seven strains of blastomyces from seven cases of Gilchrist's disease are closely related antigenically and that any one of these strains may be used as antigen in the test. This suggests that a stock strain of *Blastomyces dermatitidis* may be kept in the laboratory and used as a source of material for skin tests and complement-fixation tests. This should be of value in early diagnosis since isolation and identification of the infecting organism may require several weeks.

All the procedures described above can be carried out with materials and media present in most routine bacteriologic laboratories.

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SCHILLING'S HEMOGRAM*

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THE differentiation and enumeration of the cellular elements in a blood smear have become a highly technical aspect of clinical hematology since the publication of Arneth's "Neutrophilic Index"[1] (shift to left and right) and Schilling's "Hemogram" methods.[2]

From a study of articles pro and con in the current laboratory and clinical journals, it appears that Schilling's hemogram is favored by the majority of writers. But in spite of the fact that the hemogram has been found to be a valuable aid in clinical hematology, it seems that the method is only beyond criticism if modified or combined with an auxiliary method. It is not within the scope of this paper to discuss the various modifications suggested. It is agreed that Arneth's "neutrophilic index" is cumbersome, and that no one laboratory method should be considered absolute. However, it is my contention that the modifications of the hemogram method as presented in the current literature are not necessary if a blood smear is studied in a systematic manner.

Schilling's hemogram method has been used in this laboratory for the past five years, and in no case was it necessary to modify the method in order to detect conspicuous deviations of the differential from the normal. The reason for this lies chiefly in the early realization that only a *systematic* study of a blood smear would at all times insure a successful application of the hemogram. The method about to be described has as yet not failed to reveal what is termed by Schilling† "die biologische Reaktion einer spezifischen Krankheit."

For those who are not well acquainted with the numerical relationships of the neutrophilic elements of Arneth's and Schilling's indexes, Fig. 1 illustrates what is discussed in detail elsewhere.[3] The maturation process of the myeloid cell and its numerical appearance as a neutrophile in normal blood is demon-

*From the Anemia Laboratory, Harper Hospital, Out Patient Department.
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†Personal communication.

strated according to Schilling when 100 differential leucocytes have been enumerated, compared with the Arneth nuclear index after 100 neutrophiles have been counted. Shilling's designation of the various cell types is used.

It is essential to bear in mind that the study of a blood smear, by whatever method, is a sampling process, and that the results obtained from the sample will be valuable in direct proportion to the accuracy with which they represent conditions within the patient's peripheral blood circulation. From this point of view, the manipulation of the drop of blood from the moment it is first shed until it is ready for microscopic study is extremely important. The original cell distribution must be altered as little as possible. Furthermore, the cells actually studied, since they constitute but a fraction of those in the entire smear, must be truly representative of the smear as a whole. To insure this, a preliminary series of approximate ratios between cell types from widely separated parts of the smear is necessary; and these approximate ratios will serve as a reliable check against the final detailed results.

ANEMIA LABORATORY

Out-Patient Department, Harper Hospital

To_____Date_____

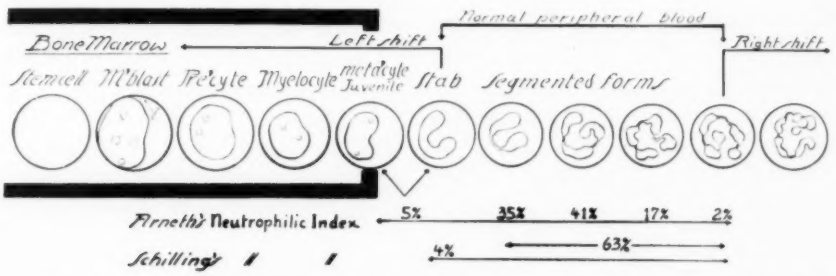

Fig. 1.—The Arneth and Schilling neutrophilic index in normal peripheral blood (left and right shift).

There are as many ways of making a blood smear and as many methods for the enumeration of the differential elements as there are laboratory textbooks. We use the so-called microslide-coverslip method for making "margin-free" smears as recommended by Schilling.[4] Only in this type of smear are the erythrocytes well spread and the leucocytes properly distributed. It is essential to keep in mind that the various leucocytes have a different degree of adhesiveness to glass, and it is, therefore, of the utmost importance to have a perfectly clean slide and coverslip. The blood film is made on the convex surface of the microslide.

Making the Blood Film.—The blood is taken from the fingertip, which is wiped off sharply with ether, not alcohol, to make it hyperemic. The fingertip is punctured about 5 mm. deep with a lancet (7 mm. long and 3 mm. in width, Eimer & Amend, New York). The first drop oozing from the puncture or upon very slight pressure is discarded (if no blood platelet count is wanted) with the aid of a clean gauze sponge. The second small drop is picked up with the edge of a No. 2 coverslip, 18 mm. size. The coverslip is put down about one-half inch from the end of the microslide at an angle of 45°. After allowing the blood to spread along the coverslip edge by adhesion, the spreader is pushed toward the

other end of the slide with an even, quick, and sure movement, so that the blood follows without any crushing. An average satisfactory preparation is made from a drop that gives a smear extending across one-third to one-half of the slide.

Although the making of a blood smear in this manner is somewhat troublesome, the flexibility of the spreader which fits the slide closely is not only a great asset in obtaining a smear of uniform thickness, length, etc., but also prevents accumulation of myeloid elements at the margin or lymphoid cells in the central part of the film.

After the smear has been properly stained and dried, it is covered with a thin film of immersion oil. The slide is then placed upon the microscope table and with the low power objective, the usable area of the blood film is determined.

Determination of the "Usable Area."—The term "usable urea" is defined as that part of the smear which shows all cells evenly distributed. The erythrocytes touch or slightly overlap each other on one extreme vertical margin and on the other they should not be more than one mean cell diameter apart.* Further, the white cell elements should be well stained to make them readily distinguishable under low power. The area about the middle part of the spread should be about 2 cm. long. With the mechanical stage vernier these extreme vertical margins (as defined above) of the area are determined and marked in order that a restudy of the area may be possible at any time.

A survey of the area is first made with the low power objective in order to gain a general idea of the relationship between the myeloid and the lymphoid elements and a knowledge of their distribution throughout the area. Then the relative average ratio is calculated. For this particular procedure the eyepiece is fitted with a device that divides the microscope field into 2, 4, or more sections.

Determination of the "Relative Average Ratio" (R.A.R.).—Within the usable area the leucocytic relative average ratio (i.e., myeloid-lymphoid ratio) is determined. The normal ratio is 2 to 3 neutrophiles to 1 lymphocyte. It must be pointed out that the myeloid part of this ratio includes also the elements coming from the reticuloendothelial system. The whole area is carefully and systematically gone over with the low power objective in such a manner that repetition of any section is avoided. About 20 to 25 individual fields which show a leucocytic ratio approximate to that gained from the general survey, are studied and the cells comprising each group (myeloid or lymphoid) are counted and tabulated in separate columns.

For example:

	MYELOID	LYMPHOID
Field 1	6	2
Field 2	12	4
Field 3	15	5

If there is any doubt as to the identity of a cell it should be determined by using the oil immersion objective. No cells which have been mechanically injured may be counted. From the data obtained, the relative average ratio per low power field is determined by adding the columns and dividing by the number of fields studied.

*This ideal "usable area" sometimes cannot be obtained when the erythrocyte concentration is less than 2 ml. per c.mm. and marked aniso-poikilocytosis is present. The vertical margins should then enclose only that area in which the red cells are not more than one and three times of the mean diameter apart.

Enumeration of the precentage relationship of the differential elements: (Schilling index.) After the relative average ratio has been determined, the actual enumeration of the percentage relationships of the differential elements, or the "internal ratios," begins. Starting at the upper edge of one vertical margin of the usable area, the low power objective is moved an entire vertical length and, keeping the ratio in mind, fields are chosen in which the ratio of myeloid to lymphoid cells approximates the relative average ratio or in other words in which the relationships of cell types approximate the average distribution of cell types in the whole area. The leucocytes in the chosen fields are counted and tabulated according to type in individual columns. The slide is moved several microscopic fields in a horizontal direction and again the objective is moved across the blood smear in the described manner, no field being studied twice.

When 200 leucocytes have been counted the myeloid-lymphoid ratio of the cells should be calculated. If care has been exercised, no difficulty will be encountered when reaching the 200 cell limit in having the "external ratio" dovetail with the relative average ratio. Although 200 cells represent the minimum number from which the percentage relationships of the differential elements may be calculated, Shilling and others recommend the counting of 300 to 400 cells. If the internal ratios (differential count) have shifted considerably after the enumeration of the second 100 cells from the number of the first 100 cells an additional 100 or 200 cells are necessary to stabilize the internal ratios. The percentage relationship of all the differential elements may be calculated by simply adding each group separately and dividing by the total number, 200, 300, etc. It does not matter how many fields or how much of the entire area has been covered if at the end of the first 200 cells the internal ratios are quite stable and the "external ratio" dovetails with the relative average ratio found by going over the entire usable area. For example, if the relative average ratio was found to be 1.6 to 1 for the usable area, the external ratio should be the same (see Table I, 1 and 2).

TABLE I

R.A.R. 1.6 to 1

NUMBER OF ENUMERATION	CELLS COUNTED	M	J	ST.	SGM.	EOS.	BAS.	MONO.	LYM.	EXTERNAL RATIO	
1	100	0	6	9	44	2	0	4	35	1.8 to 1	1.6-1
2	100	0	3	8	40	2	2	5	40	1.4 to 1	
3	100	0	4	10	40	2	1	5	38	1.6 to 1	
4	100	0	5	9	44	2	1	2	37	1.7 to 1	
Total	400	0	18	36	168	8	4	16	150	65 to 4	
	Average	0	4.5	9	42	2	1	4	37.5	1.6 to 1	

In Table I, the external ratio at the end of the final calculation dovetails at the end of 200 cells, but the internal ratio differs from the ratio of the first 100 cells. Since this is an unavoidable variation, 200 cells is the minimum number to be counted before any calculation is made.

The final percentages are reported as whole numbers. If fractions occur in either differential group, these must be absorbed first within the myeloid-lymphoid group in which they occur, by adding the fraction to that cell type that

justifies favoritism not only from a technical but also from a clinical point of view. In other words, the cell types favored or disfavored should be judged from experience gained from the study of many ratio groupings, and from the knowledge of the importance of these technical changes when the differential pattern is applied to the clinical findings.

Rule.—The first absorption is made within the myeloid-lymphoid group in which the fractions occur. Remaining fractions which cannot be absorbed within the cell types of the groups are absorbed into that main group that will either balance the external ratio (A.B., Table II) with the original relative average ratio, or will by the gain not unbalance the ratio more than ±1 per cent.

TABLE II

EXAMPLE OF ABSORPTION OF FRACTIONS
(THE RELATIVE AVERAGE RATIO IS 4 TO 1)

	PERCENTAGES AT COMPLETION OF COUNT		FIRST ABSORPTION		SECOND ABSORPTION	
Myelocytes	0		0		0	
Juveniles	5.5		6		6	
Stabs	15.5		15		15	
Segmented	52.5	A	52	80.5	52	80
Eosinophiles	3.5		4		4	
Basophiles	1.5		1.5		1	
Monocytes	2		2		2	
Lymphocytes	19.5	B	19.5	19.5	20	20

With the internal and external ratios balanced the enumeration of the differential is completed. On the final report the reticuloendothelial cells (monocytes) are grouped separately, thus:

Myelocytes	0	
Juveniles	6	
Stabs	15	
Segmented	52	78%
Eosinophiles	4	
Basophiles	1	
Monocytes	2%	
Lymphocytes	20%	

Reliable information is at hand as to the relative composition of the leucocytic pattern such as the presence of a neutrophilia or neutropenia, including the degree of "shift."

The "ratio of shift" based upon the R.A.R. is a short method for the determination of significant change within a single leucocytic group. In a case in which the precentage of a certain myeloid cell type is desired, the myeloid elements may be enumerated alone, separating the various types in the Arneth or Schilling (see Fig. 1) fashion and supplying a calculated number of lymphoid cells to make up the total of 100 cells. Or, if an enumeration of the lymphoid elements is desired as outlined by Wiseman,[5] a calculated number of myeloid elements is supplied to make up the total to 100 cells. Any group can be singled out for study by merely consulting the R.A.R. Thus, if, for example, the R.A.R. is 4 myeloid to 1 lymphoid cell, it is readily calculated that there must be 80 myeloid cells to every 100 leucocytes counted. Then going over the usable

area systematically, fields are chosen in which there is an average distribution of myeloid cell types. The myeloid cells in these fields are enumerated and tabulated according to type until the calculated number of myeloid elements has been reached. In this enumeration, any of the cell types may be still further subdivided and noted in subcolumns. Thus the "stab group" may be divided according to special morphologic characteristics into two subgroups: (A) the "band forms," which represent the normal stage in development from the juvenile to the mature segmented form; and (B) the degenerative "stab forms." In the same manner, other types of degenerative or otherwise abnormal neutrophiles may be counted separately. Again the lymphocytes are not counted, but the number required according to the ratio is supplied.

In order that the information obtained with the ratio of shift may be reliable, it is well to count two or three times the calculated number to make sure of a "shift" or the number of abnormal cell types. The greater the number of cells counted, the more certain is the stabilization of the ratios.

SUMMARY

The introduction of Arneth's "nuclear index" and Schilling's "hemogram" into clinical hematology revolutionized the method of differentiation and enumeration of the leucocytes in a blood smear.

The successful application of the differential pattern, "biologic reaction to a specific disease," depends chiefly on the method of enumeration of the leucocytes in a blood smear.

It is of the utmost importance that the applied method incorporate three features: it must (1) supply a constant that insures a uniform relative enumeration of the differential elements, (2) permit duplication of the final differential percentages at any time by any one, and therefore prevent marked fluctuation of the myeloid-lymphoid ratio that may nullify the first enumeration, and (3) insure the confidence of the clinician in the value of the leucocytic pattern as a diagnostic and prognostic aid. He may then accept instead of reject the data even though the findings deviate greatly from the expected picture.

The method described includes all of the points stressed, with the R.A.R. as the constant. Once the R.A.R. is properly determined, the method makes possible the study of different types or groups of leucocytes without the recounting of all differential cells in a blood smear. With the R.A.R. information at hand, thus changes between the myeloid-lymphoid ratio can be obtained in a short space of time.

For future reference the R.A.R. is engraved upon the glass slide or reported on the laboratory sheet. The method allows a technical error of ±1 per cent of the external ratio in relation to the relative average ratio (R.A.R.).

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A MOUSE BOX FOR OPERATING ON THE TAIL*

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IN GIVING injections intravenously into the tail of the mouse, or cutting the end of the tail in order to obtain a blood specimen, particularly when this has to be done at frequent intervals, difficulty arises when trained assistance is not at hand.

The box herein described seems to be very suitable and has given great satisfaction for many years in handling thousands of mice without assistance. Fig. 1 is self-explanatory. It consists of a slide box of the usual type, rectangular in shape, with the approximate measurements, 12 cm. in length,

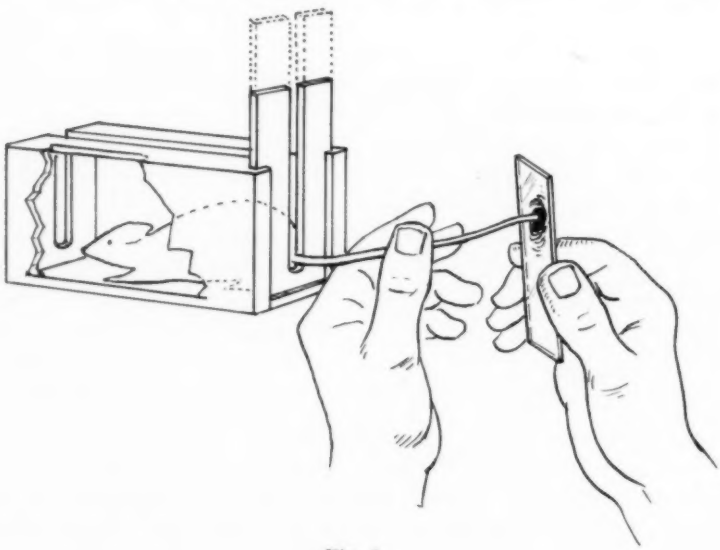

Fig. 1.

6 cm. in width, and 5 cm. in height. One end of the box is fixed, as is the roof; the other end consists of a "door" which is slid into a groove for that purpose. The end, the roof and "door" are slit sufficiently to accommodate the tail, and in such a manner that the slits are central and so continuous with one another. The mouse is allowed to enter the box, or is pulled into it by the tail, which protrudes from the slit in the roof. The sliding "door" is put in place and the tail drawn to one end of the box or the other and held on moderate tension so as to facilitate manipulation and control struggling on part of the animal. In this manner taking of specimens of blood from the tail by snipping off a piece and allowing venous oozing from the tip, or injecting material with a fine needle into the tail veins is greatly facilitated.

54 WEST SEVENTY-FOURTH STREET

*Received for publication, October 19, 1935.

USE OF THE POLAR DIAGRAM IN CHARTING INCIDENCE OF DISEASE*

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THE object in using the polar diagram in charting incidence of disease is to afford a means of more readily visualizing monthly and yearly occurrence. That this should be so is logical, since representation of occurrence of disease by a continuous line on a circular chart would throw a picture on the retina which is itself circular. There is not the abrupt discontinuity which is evident in the square and rectangular chart. In learning to tell time, individuals are schooled early in life in grasping the significance of data on the circular diagram.

The diagrams in this paper are almost self-explanatory. The total monthly incidence for the states reporting was taken from Supplements 104, 105, 109, and 112 of the U. S. Public Health Service Reports.† The zones represent the respective incidence for the years studied, the number of cases being plotted on the radii representing the months. The points for the months of each year are connected. The outer curve represents the cumulative cases for the four years. First the number of cases for 1930 were plotted. The resulting curve was then used as the base line, and upon it the cases for 1931 were plotted. This in turn was used as the base line for 1932 and so on. By this method, termed the "countour method,"[1] the lines for the years never cross. This obviates confusion of lines. When data for additional years become available, they can be plotted, using the curve of the previous year as the base line. In the diagrams shown, the months have not been corrected for the number of days. With large amounts of data, this would be necessary. Since this study is concerned with the trend of the curves and the type figure formed in the several diseases, it was not necessary to correct for population.

On observing the charts of pellagra, typhoid and paratyphoid and smallpox, it will be noted that the periods of highest incidence vary. In the diagram of pellagra you will note the hour hand is utilized to denote minimum incidence and the minute hand, maximum incidence. In my experience this emphasizes these data to a considerable extent. The curves vary in shape and in the steepness with which incidence rises and falls. Such factors for these and other notifiable diseases will be discussed elsewhere, together with variation in incidence over a period of years as illustrated on similar charts. The chart designated pellagra, smallpox, and typhoid is an example of plotting yearly

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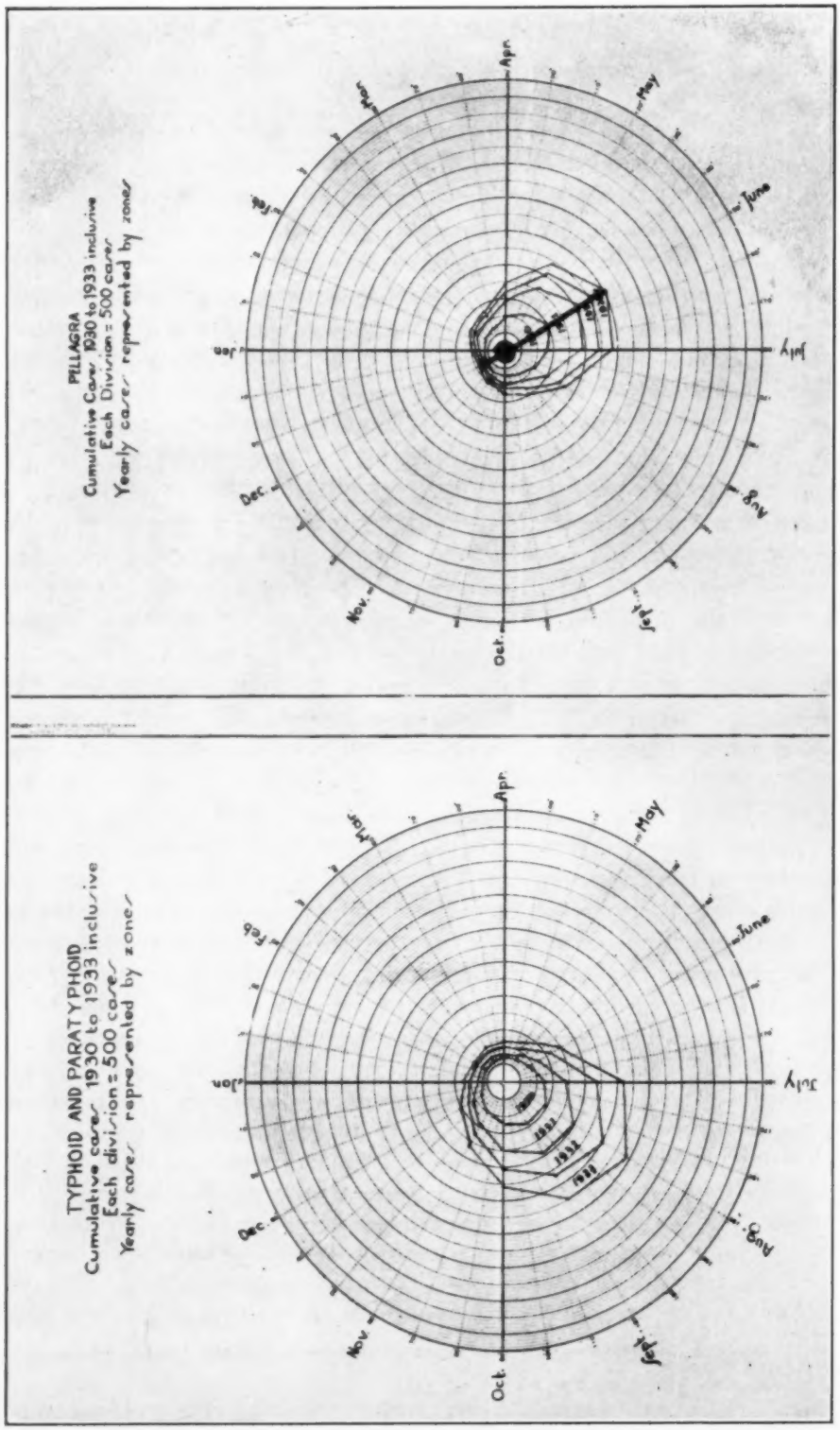
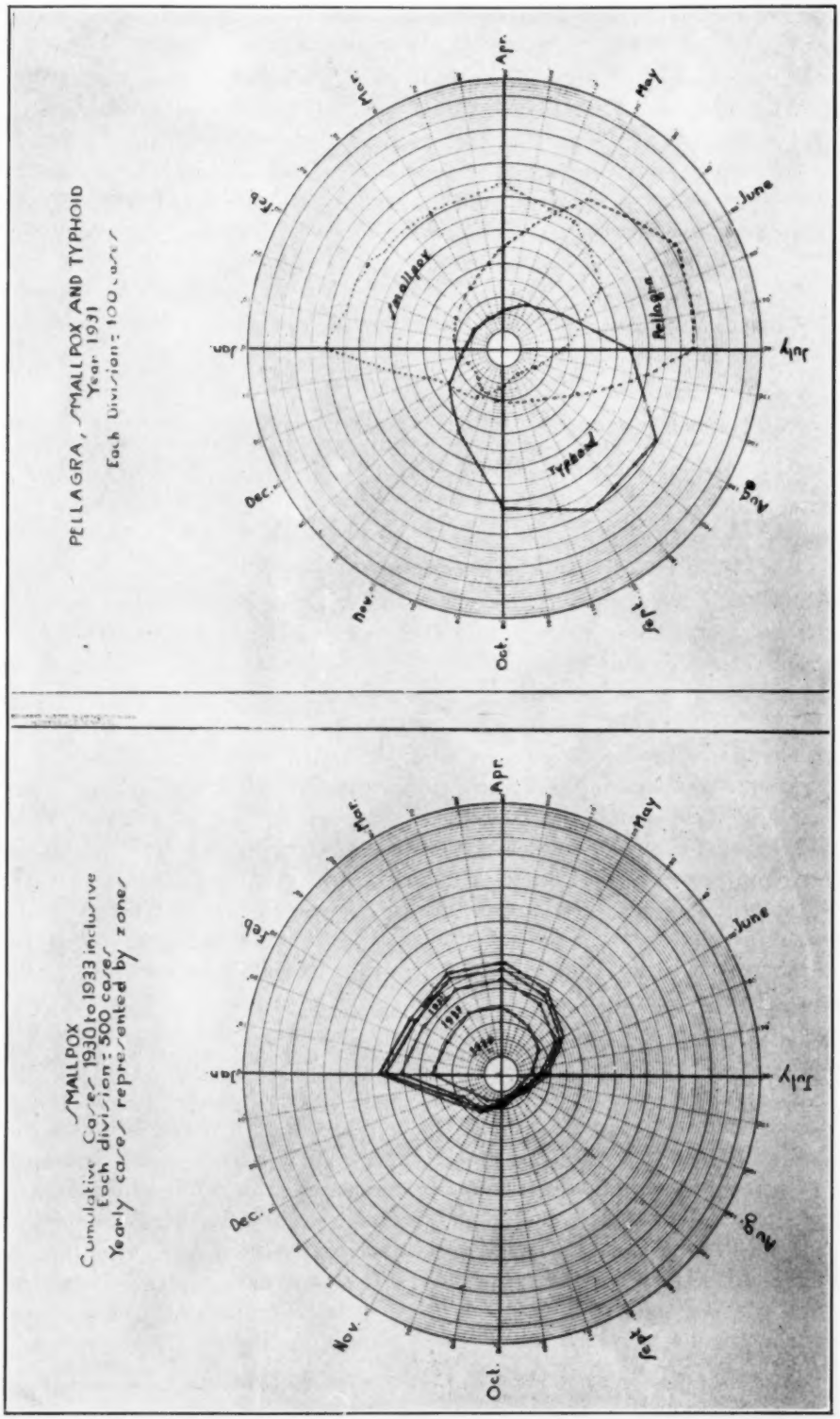
†The incidence is given as typhoid fever for the years 1930 and 1931 and as typhoid fever and paratyphoid fever for 1932 and 1933.


PELLAGRA
Cumulative Cases 1930 to 1933 inclusive
Each Division = 500 cases
Yearly cases represented by zones
Apr.
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
TYPHOID AND PARATYPHOID
Cumulative cases 1930 to 1933 inclusive
Each division = 500 cases
Yearly cases represented by zones
1930
1931
1932
1933


PELLAGRA, SMALLPOX AND TYPHOID
Year 1931
Each Division = 100 cases
Apr.
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Smallpox
Pellagra
Typhoid
SMALLPOX
Cumulative Cases 1930 to 1933 inclusive
Each division = 500 cases
Yearly cases represented by zones
1930
1931
1932
1933

incidence of three diseases on one chart. It aids in the correlation of their relative frequency and the study of the type of curve of each.

In conclusion, the polar diagram is used in statistical studies, but to my knowledge not in the manner or for the purpose illustrated here. The type of curve and the figure formed and its extent into the quadrants seems for many diseases characteristic. The simplicity of the diagrams is sufficient to illustrate incidence of the disease to the laity. The curves are extremely easy to plot, and additional data can be added for successive years.

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THE EMPLOYMENT OF OXALATED PLASMA IN THE BROMSULPHALEIN DYE RETENTION TEST*

CARL A. DRAGSTEDT, M.D., PH.D., AND MOORE A. MILLS, M.S., CHICAGO, ILL.

SINCE the development of the bromsulphalein dye retention test as a functional test of the liver by Rosenthal and White,[1] it has gained a rather widespread use because of its relative simplicity when compared to other liver function tests. During the past few years a number of workers have called attention to the fact that so-called reticulo-endothelial blockade may cause marked dye retention. In some studies on reticulo-endothelial blockade, we were desirous of making numerous bromsulphalein tests, and the need arose of making the test more rapid. It occurred to us that, if oxalated plasma could be used, the complete test could be run within fifteen to thirty minutes after obtaining the blood samples, which would greatly facilitate our work. Accordingly, duplicate tests were run on serum in the usual way and on oxalated plasma in a series of experiments to test this possibility. The plasma technic proved so uniformly satisfactory, that inasmuch as we have failed to find any suggestion of its employment in any of the current texts on laboratory and clinical methods available to us, or in the current literature, we felt the present brief report warranted.

The technic we have used is briefly as follows: The dye is injected intravenously in the usual way, 2 or 5 mg. per kilo. At five and thirty minutes samples of blood are withdrawn, using either a clean dry syringe and needle, or a syringe and needle rinsed with isotonic potassium oxalate solution. About 7 or 8 c.c. of blood are collected and introduced into a centrifuge tube containing a drop of saturated potassium oxalate, inverted once, and then centrifuged for fifteen to twenty minutes. The supernatant plasma is removed with a pipette, separated into two tubes suitable for use in the comparator box, one portion being alkalinized with sodium hydroxide. The readings are

*From the Department of Physiology and Pharmacology and the Department of Pathology, Northwestern University Medical School.

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made in the usual way. In 20 duplicate or comparison readings on serum and plasma in which the percentage dye retentions ranged from 5-0 (five and thirty minute sample) to 80-60, comparable readings were obtained in every case. As a matter of fact in addition to the considerable saving in time, the following advantages of the plasma technic became apparent; additional blood studies such as red cell counts, volume index, etc. (see Osgood et al.[2]) can be made on the same sample; and there is usually a larger amount of available plasma after centrifuging a given quantity of blood than there is serum from a similar amount, unless a considerable time is allowed for contraction of the clot.

In addition to the above, we have employed a further modification of the test which has proved serviceable, whenever, for example, shortly after an intravenous injection of India ink for purposes of blockade, or in the accident of some hemolysis, the plasma is not perfectly clear. This consists of precipitating the proteins in the plasma with two volumes of acetone, centrifuging for five minutes and making the dye reading by alkalinizing the clear supernatant fluid. This technic is used in the rose bengal test. Allowance is made for the dilution and the readings made in the usual way. In our hands, the readings after acetone precipitation have tended to be about 5 per cent lower than corresponding readings on the unchanged plasma when the retention is marked. Traces of dye are, however, very readily recognized by this procedure. This technic is not suitable for routine use.

In conclusion, we have found the use of oxalated plasma for the bromsulphalein retention test accurate, simple and rapid, less likely to fail than the serum technic, and advantageous in that other blood studies can be made on the same samples of blood used for the test. In addition, acetone precipitation of the plasma proteins has been found to be efficacious in clearing the plasma of ink and other blockading agents, enabling the test to be run immediately after blockading procedures.

NOTE.—Since submitting this paper for publication, it has come to our attention that Pratt and others[3] have used plasma samples for bromsulphalein dye readings. No record of comparison with identical serum samples is given.

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AN IMPROVED SYSTEM OF FILING SURGICAL REPORTS AND SLIDES*

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BECAUSE of the steady increase in the number of blocks being put through from each surgical specimen, we have been obliged to change our filing system from that of the card with the slide attached to the present one which consists of an envelope containing a slide holder, and copy of the report (Fig. 1).

The envelope is a standard commercial one measuring 4 inches by 6¼ inches, made of glazed laid kraft paper, weighing 62 pounds to the ream, and open at one end. The gummed flap is cut off leaving a plain open end.

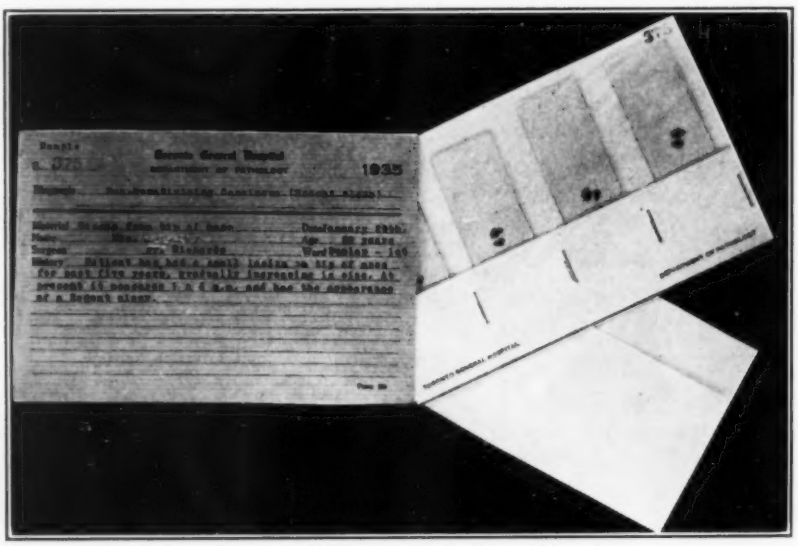

Fig. 1.

The slide holders are made of heavy grayish white sulphite tag, weighing 400 pounds to one thousand sheets of 24 inches by 37 inches. These are cut to 5¼ inches by 5⅞ inches. A black line is printed 1½ inches from the bottom to mark the line of fold. At the same time is printed guiding marks for the stapling as well as the hospital name. After folding the card on the black line, five staples are inserted as indicated in Fig. 1, thus making a card 3¾ inches by 5⅞ inches with pockets to hold four slides. This fits quite easily into the 4 inch by 6¼ inch envelope. At the upper left-hand corner of this slide holder, the year is printed with a rubber stamp. On the upper right

*From the Department of Pathology, Toronto General Hospital.
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hand corner, with an automatic numbering machine, the particular number of the surgical specimen is stamped. The envelope will accommodate three of these slide holders, thus taking care of twelve microscopic sections, besides leaving room for a copy of the report, drawings, and photomicrographs.

The envelope with its contents is now filed in serial order in a steel cabinet of drawers to hold 4 inch by 6 inch cards. They are indexed under the surgeon's name, the patient's name, and also in a disease and region file. For this indexing the Kardex visible records system serves excellently.

THE USE OF LIQUID AIR IN COOLING KNIVES AND OF GELATIN FOR MOUNTING IN FROZEN SECTION TECHNIC*

S. CARL WERCH, M.D., ELOISE, MICH.

TWO small changes which appear to be improvements in the usual frozen section technic have been put into operation. The first is to immerse the microtome knife in liquid air in a thermos bottle immediately before using. It takes five minutes to warm up to thawing temperature and has simplified the procedure. Liquid air temperature has no damaging effect on the knife.

The second point is the use of 10 per cent gelatin solution instead of glycerin for mounting. Fewer air bubbles are trapped with this method.

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*From the Eloise Hospital and Infirmary and the University of Illinois College of Medicine, Department of Physiology.

Received for publication, October 26, 1935.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

THYROID FUNCTION, Total Blood Fat Determination as an Index of, Chamberlain, C. T., Jacobs, S., and Butler, M. F. Am. J. M. Sc. **191:** 67, 1936.

The authors do not suggest that determination of the total lipoids should supplant any method used at the present for the study of metabolic disorders; rather it is offered as a valuable addition. They feel that it will afford as much information as will determinations of plasma cholesterol in that it is consistently elevated above the normal 700 mg. per 100 c.c. in hypofunction of thyroid and generally below 400 mg. in hyperthyroidism. In mild cases of hyperfunction it is apt to be between 400 and 500 mg. at which level the diagnostic import is questionable. It is not materially affected by any other endocrine gland disorders, except diabetes, as long as the thyroid is not demonstrably involved.

It is exceptionally of value in those patients for whom the determination of the basal metabolic rate is not feasible.

The depression of the basal metabolic rate following administration of iodine, such as Means has shown to be a routine diagnostic test for hyperthyroidism, is paralleled by the increase of total lipoids. Conversely, its failure in response to iodine is regarded as of significant negative value.

CREATINURIA, of Infancy and Childhood, Marples, E., and Levine, S. Z. Am. J. Dis. Child. **51:** 30, 1936.

A study of physiologic creatinuria and the influence of extraneous factors on such creatinuria was made on eight normal infants aged from three weeks to seven months. The composite data were collected from a total of one hundred and twenty-nine days. The results may be summarized as follows:

All the infants excreted creatine in the urine, the creatine coefficients (mg. of nitrogen per kilogram of body weight in twenty-four hours) varying from 0.3 to 5.14. The average value for infants over one month of age was 3.89. The coefficients ranged from 3.74 to 5.78, with an average of 4.93. The total creatine coefficients (creatinine plus creatine) ranged from 4.82 to 10.5 with an average of 8.86 for the group of infants over one month of age. With few exceptions the total creatine coefficients fell within the range of coefficients for preformed creatinine established for adults.

Age appeared to have no influence on the excretion of creatinine within the limited age range studied here (three weeks to seven months). Premature infants excreted practically no creatine, and this was also true for some very young full-term infants.

Creatinuria of infants could be diminished by feeding diets low in protein but of adequate caloric value. It could be increased by feeding diets high in protein. The excretion of creatinine was not materially affected by these changes in diet.

Infants have a low tolerance for ingested creatine. From 55 to 65 per cent of the amount ingested was excreted in the urine as creatine in the first twenty-four hours and 63 to 82 per cent in forty-eight hours.

The ingestion of amino-acetic acid increased the spontaneous creatinuria of infants but had no effect on the excretion of creatinine. Only a small fraction of the ingested amino-acetic acid was represented in the extra creatine excreted, and there was no specific quantitative relationship between the amount of amino-acetic acid ingested and the extra creatine excreted.

The effect of withdrawal of available amino-acetic acid from the body by diverting it to the formation of hippuric acid for detoxification of benzoic acid was studied. The ingestion of sodium benzoate causes an initial increase in creatinuria, due perhaps to the increased protein metabolism caused by the benzoate, followed by a decrease to below the foreperiod level.

CEREBROSPINAL FLUID, Comparative Dextrose Content of Lumbar and Cisternal, Levinson, A., and Cohn, D. J. Am. J. Dis. Child. **51**: 17, 1936.

A study has been made of the comparative dextrose content of the lumbar and the cisternal cerebrospinal fluid obtained from nonmeningitic and meningitic patients and from normal dogs.

In nonmeningitic patients the dextrose content of the cisternal fluid was found to be either equal to that of the lumbar fluid or slightly higher. The dextrose content of the lumbar fluid of those patients in whom no pathologic change in the nervous system was found averaged 57 mg. per hundred cubic centimeters, and that of the cisternal fluid, 60 mg. The results on normal dogs were similar to those on human beings.

In patients with tuberculous meningitis and in untreated patients with meningococcic meningitis, the dextrose content of the lumbar fluid was always reduced. The dextrose content of the cisternal fluid, while usually lower than normal, was not as low as that of the lumbar fluid. In some instances the dextrose content of the cisternal fluid was within the normal range, even though dextrose was absent or very little was present in the lumbar fluid. In patients with meningococcic meningitis after serum treatment the values for dextrose in the cisternal and lumbar fluid were much closer to the normal than the values found for untreated patients, and the differences between the levels in the two fluids were smaller.

In the majority of a limited series of fluids from patients with nonmeningococcic purulent meningitis, particularly of the pneumococcic and streptococcic types, there was a very low amount of dextrose or none in the cisternal as well as in the lumbar fluid.

Determinations of the dextrose content of the lumbar fluid are more reliable for the diagnosis of meningitis than determinations made on the cisternal fluid because the dextrose content of the cisternal fluid is seldom reduced as greatly as that of the lumbar fluid.

TUBERCLE BACILLUS, A Glycerol-Free Medium for, Henley, R. R. Am. J. Tuberc. **32**: 724, 1936.

During the course of studies of the biology of the tubercle bacillus in these laboratories, a medium has been devised which contains neither amino-acids nor glycerol, but which nevertheless affords an abundant growth of the human type of the organism. Since a medium of this type may be of value to those engaged in the study of the metabolic processes of the tubercle bacillus the following notes are presented:

Ammonium malate	12.53 gm.
Dipotassium phosphate	1.80 gm.
Sodium citrate	0.75 gm.
Magnesium sulphate	1.50 gm.
Ferric citrate, scales, U.S.P. VIII	0.30 gm.
Dextrose, of the grade known as Cerelose	80.00 gm.
Water, distilled, to make	1,000.00 c.c.

PREPARATION OF MEDIUM

The ammonium malate is prepared by dissolving 10 gm. of malic acid (grade known as "Food Pure") in about 500 c.c. of distilled water. Ammonium hydroxide, C.P., is added to the solution until this is neutral to litmus. The other ingredients of the medium are then added in the amounts and order given in the formula. Each compound is dissolved completely before the next is added. The required amount of ferric citrate is dissolved in hot water before it is added to the solution.

The completed medium is placed in 300 c.c. Erlenmeyer flasks, 100 c.c. per flask, and is sterilized by heating under 10 to 12 pounds' steam pressure for twenty minutes, or, alterna-

tively, by heating in flowing steam for thirty, twenty, and fifteen minutes, respectively, on each of three successive days. Heating causes a lowering of the pH value; hence, excessive heating must be avoided. The pH value of the sterilized medium should not be lower than 6.0. It is possible and desirable so to prepare and sterilize the medium that the pH will be not lower than 6.4 nor higher than 7.

The sterilized medium is inoculated with seed from vigorously growing cultures not more than fourteen days old, and the inoculated medium is incubated at 37.5° C.

Growth starts slowly, requiring four weeks or longer to reach 0.50 gm. dry weight of bacilli per 100 c.c. of culture medium, but, once started, it continues steadily and reaches a maximum about the eighth week. The greatest weight thus far obtained is 1.82 gm. per 100 c.c. culture fluid, and the average weight of bacteria in mature cultures is approximately 1.60 gm. per 100 c.c. As with other media, the weight of bacteria reaches a maximum and then tends to decrease. Growth is not always uniform, since differences in weight are frequently found between two or more cultures of the same age and batch.

BLOOD, Puncture of Spleen in Diseases of, Perles, S. Paris méd. **25**: 217, 1935.

The following is the normal "splenogram":

Neutrophilic polynuclears	20 to 30%
Eosinophilic polynuclears	1%
Basophilic polynuclears	rare
Typical lymphocytes	50 to 60%
Medium mononuclears	10 to 20%
Monocytes	5 to 10%
Plasmocytes	1 to 2%
Normoblasts	0.20%
Naked nuclei	numerous
Background, thick, sometimes granular	
Erythrocytes, normal	
Platelets, very numerous	

ANEMIA, Absorption, Storage, and Elimination in Experimental, Hahn, P. F., and Whipple, G. H. Am. J. M. Sc. **191**: 24, 1936.

Reserve iron storage in the dog can be exhausted during two to three months by a continuous anemia with hemoglobin level maintained at one-half to one-third of normal. Given this elimination of reserve iron we may attempt to trace iron given by mouth or vein.

A rapid turnover of iron is the conspicuous feature of the experiments dealing with iron given by mouth and short feeding experiments (one to two days) give no evidence of any iron store in the liver. As the iron feeding experiments are lengthened we see a variable accumulation of iron in the liver, but the amounts are small and a very rapid appearance of the iron in matured hemoglobin is the conspicuous feature.

The importance of blood-free organs is stressed, and other method difficulties are discussed.

Parenchyma iron of various blood-free organs is relatively a constant in these dogs. Liver, kidney and pancreas average 1 to 2 mg. Fe per cent. The lung is a little higher, or 3 mg. Fe per cent. The spleen is still higher, or 5 to 6 mg. Fe per cent. The red marrow apparently has the highest level of parenchyma iron, an uncertain figure but probably in excess of 10 mg. Fe per cent. The striated muscles actually rate with the liver and kidney, although the average for total contained iron is 3.1 mg. Fe per cent. About 1.6 mg. Fe is muscle hemoglobin iron, leaving the parenchyma iron as 1.5 mg. Fe per cent.

Muscle hemoglobin iron and muscle parenchyma iron are inviolate stores of iron which are not drawn upon no matter how great is the emergency due to anemia. Conversely, no surplus iron can be demonstrated in this tissue where iron is given intravenously.

Iron depletion can be carried to a point where there is almost a complete cessation of hemoglobin production in a standard dog on a diet very poor in iron.

Intravenous iron in the doses given will result in large storage in the liver and spleen, 55 to 70 per cent of the total Fe given. The authors are not prepared to say where the remaining iron is to be located in the body tissues or fluids but it certainly is not eliminated.

ANEMIA, Iron and Its Utilization in Experimental, Whipple, G. H., and Robscheit-Robbins, F. S. Am. J. M. Sc. **191**: 11, 1936.

Iron given intravenously to these standard anemic dogs, in large or small doses, will be returned quantitatively as newformed hemoglobin on the basis of 10 mg. Fe – 3 gm. hemoglobin.

When iron is given by mouth to these standard anemic dogs the influence on hemoglobin production is not proportional to the amount of iron administered. An optimum dose of iron (40 mg. Fe per day for two weeks, or 560 mg.) will give a net output of about 55 gm. hemoglobin, and this means about a 35 per cent utilization of the iron given. With larger doses the hemoglobin output increases a little, and with 400 mg. Fe daily the net output would average about 95 gm. hemoglobin, or about a 5 per cent utilization of the iron given by mouth.

Iron contained in salmon bread under certain conditions may be utilized by these anemic dogs up to about 40 per cent to produce hemoglobin. Iron in the normal liver when fed may show a 45 per cent utilization of this tissue iron.

When liver feeding is combined with iron feeding, there is a definite summation effect.

When intravenous iron in large doses is combined with liver feeding or iron by mouth, there is no evidence of summation.

This probably means that the top functional capacity of the body to produce hemoglobin has been reached, about 10 gm. hemoglobin output per day.

Iron salts are utilized with equal facility by the dog to produce hemoglobin when given by mouth in the ferrous, ferric or reduced state. The determining factor is the amount of the iron metal.

The Eck fistula and splenectomy lessen the capacity of the anemic dog to conserve and utilize iron given by vein to produce new hemoglobin.

SILICOSIS, Clinical Observations on the Pulmonary Blood Flow in, and Other Fibrotic Conditions of the Lungs, Miller, H. R. Am. J. M. Sc. **191**: 334, 1936.

The fibrosis in pulmonary disease assumes two main forms: (a) A diffuse type involving the vast capillary alveolar bed, associated, as a rule, with pulmonary hypertension and observed in emphysema or in obstruction to the lesser circulation (mitral stenosis, kyphoscoliosis, etc.), and (b) a circumscribed or "patchy" fibrosis involving the parenchyma and encountered in tuberculosis, carcinoma, etc. In silicosis the diffuse type is present early and the second, or patchy, form appears much later.

From a vascular point of view the heart and lungs are one organ; the lungs represent an enormous vascular sponge of ramified vessels stemming from the heart and surrounded by air sacs through which gases are exchanged between the circulation and the outside atmosphere. With this concept in mind it is not surprising to find that pulmonary disease and conditions associated with marked fibrosis remain extravascular, as a rule, and that when the peripheral vascular lung bed becomes involved a concomitant state of pulmonary hypertension is the rule. The heart, if at all affected, is drawn into the picture secondarily, either (a) by developing hypertrophy of the right ventricle as a response to this hypertension, in some cases accompanied by the terminal picture of so-called right heart failure; or else (b) the heart responds by altering its volume output as a compensatory reaction to an increase in the A-V difference, this difference following as a result of an interference in gas exchange through a pathologically altered alveolar wall.

Unless cardiac failure (congestive failure) intervenes, the rate of pulmonary blood flow remains normal in both arterial and venous circuits in all pulmonary fibrotic conditions. To

this there are few exceptions. As in other fibrotic conditions, the rate of pulmonary blood flow in silicosis is not deranged unless heart failure sets in.

The rate of pulmonary blood flow is retarded in the venous channels in the presence of cardiac congestive failure.

In a few instances of pulmonary fibrotic states the velocity of blood flow was retarded in the pulmonary arterial channels. Here the fault would appear to be in the alveolar membrane.

CANCER, The Reaction of Spontaneous Mouse Tumors to Cystine Disulphoxide, Staff of Lankenau Hospital Research Institute. Am. J. Cancer 26: 554, 1936.

The daily subcutaneous administration of 0.0085 gm. cystine disulphoxide to mice with spontaneous tumors was accompanied by tumors of lesser maximum size than those in untreated controls, by a lesser maximum percentage increase in volume, by a slower attainment to a given volume, and by a lesser percentage of tumors attaining a given volume. The tumors of the disulphoxide injected mice were watery and diffuse; they had fewer cells per unit measure and their nuclei tended to be larger than those of controls. The anemia of malignancy tended to be enhanced. Body weights were essentially unaffected. A tendency for the injected mice to live longer than the controls was exhibited. There was no overt tissue pathology.

The consistency in and between these data leads to the conclusion that proliferation growth of malignant spontaneous tumors of the mouse is retarded, as is that of all other expressions so far encountered, by partially oxidized derivatives of the sulphhydryl group as here represented by cystine disulphoxide.

Correspondence

Two twelve-month Residenceships in Allergy are available with Doctors Vaughan and Graham in Richmond, Virginia, for properly qualified young physicians. Appointments are made semiannually. Completion of an interneship, either rotating or in Internal Medicine in a nationally recognized teaching hospital, is a prerequisite.

The course of training includes practical experience in the study and treatment of the allergic diseases as well as general training in the practice of internal medicine. Rooms and offices are provided but not board or laundry. In lieu of the latter, there will be a salary of seventy-five dollars per month.

Residents will be required to devote a portion of their time to investigative work (clinical and laboratory) in the field of allergy.

Inquiries or applications should be made to Dr. Warren T. Vaughan, 201 West Franklin Street, Richmond, Va.

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